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Proceedings

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United States Papers

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PROCEEDINGS

TENTH UNITED STATES/JAPAN CONFERENCE ON SEWAGE TREATMENT TECHNOLOGY

OCTOBER 17-18, 1985

AND

NORTH ATLANTIC TREATY ORGANIZATION/COMMITTEE ON THE CHALLENGES OF MODERN SOCIETY (NATO/CCMS) CONFERENCE ON SEWAGE TREATMENT TECHNOLOGY

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VOLUME I.

PART B. UNITED STATES PAPERS

U.S. ENVIRONMENTAL PROTECTION AGENCY
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NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

The maintenance of clean water supplies and the management of municipal and industrial wastes are vital elements in the protection of the environment.

The participants in the Japan-United States-North Atlantic Treaty Organization/Committee on the Challenges of Modern Society (NATO/CCMS) Conferences on Sewage Treatment Technology completed their conferences in Cincinnati, Ohio, in October 1985. Scientists and engineers of the participating countries were given the opportunity to study and compare the latest practices and developments in Canada, Italy, Japan, The Netherlands, Norway, the United Kingdom and the United States. The proceedings of the conferences comprise a useful body of knowledge on sewage treatment which will be available not only to Japan and the NATO/CCMS countries but also to all nations of the world who desire it.

Lee M Thomas

Lee M. Thomas
Administrator

Washington, D.C.

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16. ABSTRACT

This publication is one of three volumes that contain Japanese, American, and NATO papers presented at the Tenth United States/Japan Conference on Sewage Treatment Technology and the North Atlantic Treaty Organization/Committee on the Challenges of Modern Society (NATO/CCMS) Conference on Sewage Treatment Technology held in Cincinnati, Ohio, October 1985. The proceedings of the conferences comprise a useful body of knowledge on advances in sewage and wastewater treatment and sludge management technology. Subject matter covered includes municipal wastewater treatment including bioengineering applications and anaerobic treatment; sludge treatment and disposal including combustion, oxidation, composting, and toxics removal; odor control; small flow sewerage system; sludge conversion to oil, etc. Volume I, Part A, contains the Japanese Papers; Volume I, Part B, contains the American Papers; and Volume II contains the NATO Papers.

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VISIT TO THE MULTIPLE DIGESTION PROJECT,
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JOINT COMMUNIQUE

TENTH UNITED STATES/JAPAN CONFERENCE
ON SEWAGE TREATMENT TECHNOLOGY

Cincinnati, Ohio
October 18, 1985

1. The Tenth United States/Japan Conference on Sewage Treatment Technology was held in Cincinnati, Ohio, from October 17 to 18, 1985.

2. The Japanese delegation headed by Dr. Takeshi Kubo, Counselor, Japan Sewage Works Agency, was composed of two representatives from the Ministry of Construction, three representatives from the Japan Sewage Works Agency and one each from the local governments of Tokyo, Yokohama and Fukuoka.

3. Mr. John J. Convery, Director, Wastewater Research Division, Water Engineering Research Laboratory, U.S. Environmental Protection Agency, was head of the U.S. delegation, which consisted of seven representatives of the federal government, five academia representatives and three representatives from consulting engineering firms and scientific laboratories.

4. The chairmanship of the Conference was shared by Mr. John J. Convery and Dr. Takeshi Kubo.

5. During the Conference, papers relating to the joint research projects on sludge treatment and disposal, including combustion, oxidation and composting, were presented by both sides. Data and findings on the joint research projects were mutually useful and provided increasing insights into the nature of the problems and potential solutions for each country. A decision was made to expand the scope of the joint research projects to include anaerobic treatment of wastewater.

6. Principal topics of the Conference were bioengineering applications in wastewater treatment as well as sludge management and disposal, aeration practice, wastewater reuse, odor control, small flow sewerage system, nutrient control and innovative biological treatment processes.

The discussions which followed the presentations were also useful to both countries.

7. Field visits in Lawrence, Marlborough and Hartford, Connecticut; Chicago, Illinois; Madison and Milwaukee, Wisconsin; and Sacramento, California; are planned to inspect wastewater treatment facilities in these areas.

8. Recent engineer exchanges between the two countries included a two-week visit in 1985 to Japan by Mr. James F. Kreissl, Wastewater Research Division, Water Engineering Research Laboratory, U.S. Environmental Protection Agency, and a fourteen-month visit to the United States by Dr. Kazuhiro Tanaka, Japan Sewage Works Agency, in 1984 to 1985. Mr. Takashi Kimata of the Japan Sewage Works Agency is now staying at the above U.S. EPA Cincinnati Research Laboratory. Both parties agreed to continue the engineer exchange program.

9. It was proposed by the Japanese side that the Eleventh Conference be held in Tokyo, Japan, about September 1987, and the future Conferences in the United States would be held in Cincinnati, Ohio and also in Washington, D.C. as were the past Conferences.

10. A proceedings of the Conference will be printed in English and Japanese.

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STRUCTURE OF METHANOGEN GENES

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This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

Prepared for Presentation at:

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on Sewage Treatment Technology

October 17-18, 1985
Cincinnati, Ohio

STRUCTURE OF METHANOGEN GENES

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ABSTRACT

Methanogens are members of the third biological kingdom known as the Archaebacteriales. Their sizes and cellular organizations are typically prokaryotic whereas biochemical and molecular biological analyses have demonstrated the presence of eukaryotic properties. Our studies have two goals; to establish the structure and mechanisms of expression of methanogen genes and to apply this knowledge to the manipulation of genes which encode enzymes directly involved in methane biogenesis. We have cloned and sequenced several methanogen genes which, when expressed in *Escherichia coli*, complement auxotrophic mutations of this laboratory prokaryote. Analyses of the DNA sequences indicate that methanogen genes are organized into multigene transcriptional units and that translation of mRNAs employs ribosome binding sequences. These are properties typical of eubacteria; in contrast, there is no evidence for eubacterial promoter-like sequences and comparisons of methanogen and other archaebacterial sequences indicate that archaebacterial promoters resemble the sequence used in both yeast and in the fruit-fly (*Drosophila*) to direct the transcription of heat-shock genes. An analysis of the structure and presumed regulatory signals in sequenced methanogen genes is presented in this paper.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

INTRODUCTION

Methane biogenesis ranks with processes such as photosynthesis and nitrogen fixation as a major factor in the global cycling of biochemicals. Methane generating micro-organisms (methanogens) play a vital role in the world's environment by catalyzing the final stage in the decomposition of waste biomass to methane. As the opportunities to use methane as a fuel increase it is to be expected that methanogens may also soon play a much larger role in the world's economy. The study of methanogens themselves has been impeded by their extreme sensitivity to oxygen; they require redox potentials below -330mV for growth and only recently have techniques been developed by which methanogens can easily be cultivated as pure cultures in the laboratory (1, 2). The advent of these techniques has facilitated a rapid expansion in the study of the biology of methanogens and the biochemistry of methane biogenesis. Results obtained to date indicate that methanogens form an extremely diverse group (1, 3, 4 are reviews). They span the full range of prokaryotic morphological types and have DNAs with base contents ranging from 27.5 to 52% G+C. It has, in fact, been proposed that methanogens are not true prokaryotes but are representatives of a third biological kingdom called the Archaebacteriales (1, 5). This kingdom, which includes extreme halophilic and acido-thermophilic micro-organisms in addition to the methanogens, was proposed because its members have several properties radically different from both prokaryotic and eukaryotic species. Archaebacteria have unique structural subunits in their cell envelopes and lipids (6). Their RNA and protein synthesizing systems are resistant to most of the antibiotics which inhibit these processes in prokaryotes and eukaryotes (2, 7, 8). Their DNA-dependent RNA-polymerases are very different from those of classical prokaryotes (9) and comparative analyses of the sequences of tRNAs and rRNAs have provided convincing evidence that whereas the different archaebacterial types are related to each other they are only very distantly related, in evolutionary terms, to current prokaryotic and eukaryotic species (1). It has recently been shown that some archaebacterial tRNAs contain intervening sequences, introns, a property generally associated with eukaryotic but not prokaryotic species (10). Our studies have focused on cloning and analysis of polypeptide encoding genes. We have been able to clone fragments of DNA, from several different methanogens, which function in E. coli to complement auxotrophic mutations in this eubacterial species (11-14). This procedure has allowed us to identify and subsequently obtain the nucleotide sequences of the methanogen genes which function in E. coli. The accumulated data has provided the first description of the organization of protein encoding genes within the genomes of methanogenic archaebacteria. This information is presented in detail.

RESULTS AND DISCUSSION

Genes cloned from methanogens. Table 1 contains a list of cloned methanogen genes which function in *E. coli*. Expression of these genes results in complementation of the listed auxotrophic mutations. Additional genes have been cloned whose functions are unknown but which can be identified as open reading frames (ORF's; 11-13). Methanogen genes cloned by other research groups encode sub-units of the enzymes methyl-coenzyme-M methyl-reductase and DNA-dependent RNA-polymerase (15, 16) and encode tRNAs and rRNAs (17, 18).

TABLE 1. CLONED METHANOGEN GENES WHICH COMPLEMENT AUXOTROPHIC MUTATIONS IN *E. COLI*

Mutation Complemented[*]	Methanogen DNA	Size of encoded Polypeptide (Kd)	Reference
hisA[†]	M. voltae	26	11.
hisA	M. vannielii	26	11.
argG	M. voltae	55	19.
argG	M. vannielii	51	Unpublished [illegible]esult
argG[‡]	M. barkeri	51	14.
proC	M. smithii	28	12.
purE$_1$	M. smithii	37	12.
purE$_2$	M. smithii	37	12.
purE$_1$	M. thermoautotrophicum	36	13.
purE$_2$	M. thermoautotrophicum	36	13.

[*] Cloned methanogen DNAs complement mutations in the listed *E. coli* genes.

[†] The *M. voltae* DNA also complements mutations in the *hisA* locus of *Salmonella typhimurium* (11).

[‡] The *M. barkeri* DNA also complements mutations in the *argA* locus of *Bacillus subtilis* (14).

Methanogen gene organization. Methanogens are archaebacteria (1) and our current experiments are aimed at determining whether the structure and expression of methanogen genes follow prokaryotic or eukaryotic principles or whether archaebacteria have genetic arrangements different from both eukaryotes and prokaryotes. The following paragraphs address molecular biological parameters which are either typically prokaryotic or typically eukaryotic.

a) Genetic code and introns. The demonstration that cloned methanogen genes can direct the synthesis of functional enzymes in *E. coli* (Table 1) strongly implied that these genes employed the universal genetic code and did not contain introns. DNA sequencing has confirmed this prediction (11-13). It should be noted however that there have been many unsuccessful attempts to clone methanogen genes which would complement mutations in additional *E. coli* genes. These unsuccessful attempts are, of course, not listed in Table 1 and it should be recognized that the procedure used to obtain the methanogen genes listed in Table 1, i.e. functional expression in *E. coli*, presumably would select against the isolation of methanogen genes employing an unusual genetic code or which contained introns. Analysis of DNA sequences surrounding cloned genes, sequences whose functional expression was not demanded in *E. coli*, might allow the recognition of introns in polypeptide encoding sequences. One such candidate sequence has already been identified although the evidence for this sequence being an intron is currently only circumstantial (11).

b) Operons and ribosome binding sites. Prokaryotic genes are frequently contained in polycistronic transcriptional units or operons. In contrast, eukaryotic genes are not usually transcribed into polycistronic mRNAs. Parameters expected of genes in an operon are that mutations in promoter proximal genes are polar on expression of promoter distil genes, only short intergenic sequences are present between genes in the same operon and ribosome binding sequences are positioned immediately preceding the AUG initiation codon of each polypeptide-encoding gene. These properties have been demonstrated for two genes on a fragment of DNA cloned from *M. smithii* which complements *purE* mutations in *E. coli* (12). Only one of the cloned genes is needed for *purE* complementation although both genes direct the synthesis of polypeptides in *E. coli*. Polycistronic mRNAs must contain signals to correctly position ribosomes for initiation of translation at the start of genes embedded within the polycistronic mRNA. Ribosome binding sites contain sequences which are complementary to a sequence found near the 3' terminus of the 16SrRNA molecule (20). Table 2 shows that ribosome binding sites precede the sequenced methanogen genes.

The data from DNA sequencing which indicate that methanogen genes are organized into prokaryotic-like transcriptional units and encode prokaryotic-like mRNAs are supported by a study of the structure of *M. vannielii* mRNAs (21). *M. vannielii* mRNAs are unstable, have only short 3' polyadenylation tracts and do not contain 5' capped nucleotides; all features of prokaryotic but not eukaryotic mRNAs.

TABLE 2. RIBOSOME BINDING SEQUENCES PRECEDING METHANOGEN GENES

Methanogen	Gene[†]	Sequence[‡]
Methanobrevibacter smithii	ORF-Pur$_s$	** **** * AGAAGGTATTTTAAAATG
Methanobrevibacter smithii	ORF-B$_s$	* *** ATAAGGGATAATTATG
Methanobrevibacter smithii	ORF-ProC	* ** **** AAGATGTGAAATATATG
Methanobrevibacter smithii	ORFIS	**** * GTGAGGACAAATAATATTTTATG
Methanobacterium thermoautotrophicum	ORF-PurE$_t$	****** TAAAGGTGAATCTCCAGATG
Methanobacterium thermoautotrophicum	ORF-B$_t$	* ****** TGATGGTGATTGAATG
Methanobacterium thermoautotrophicum	ORF-C$_t$	** ***** AGAAGGTGTACTGATG
Methanococcus vannielii	ORF-HisA	****** AAAAGGTGAATACAATG
Methanococcus vannielii	ORF-76	****** TTCTGGTGATTCAATG
Methanococcus voltae	ORF-HisA	* ***** AGATGGTGAAACTGATG

[†] The sequence data and gene designations are taken from publications 11-13.

[‡] A sequence exactly complementary to 3' end of the 16SrRNA of *M. smithii* and *M. thermoautotrophicum* would be 5'AGGAGGTGAT. The 16SrRNA of methanococci lacks the 3' terminal U and therefore the complementary sequence would be 5'GGAGGTGAT (1). The asterisks (*) indicate bases in the methanogen sequences which are complementary to the 16SrRNA. The initiation ATG codon of each polypeptide encoding gene is underlined. The number following the base in the sequence $A_7G_9G_{10}T_8G_7A_7$ is the number of times that base occurs in the location indicated in the 10 sequences listed in Table 2. We propose this sequence as in the consensus ribosome binding sequence in methanogens.

c) *RNY rule*. Shepherd (22, 23) has proposed that polypeptide-encoding ORFs can be recognized by the use of RNY codons (R=purine, Y=pyrimidine, N=purine or pyrimidine). RNY codons occur most frequently in the correct reading-frame whereas non-utilized ORFs do not show preferential RNY codon usage. The only reported exception to this rule, other than the highly evolved overlapping genes of bacteriophages such as ϕX174, is the archaebacterial gene of *H. halobium* which encodes bacterio-opsin (23). We have now analyzed the available archaebacterial genes from methanogens, both those known to encode functional polypeptides and those only identified by computer screening as ORFs, for RNY codon usages. Table 3 shows that the majority of methanogen-derived genes follow the RNY rule. There are two exceptions, ORF-D of *M. smithii* and ORF-*PurE*$_t$ of *M. thermoautotrophicum*. ORF-D is encoded on the DNA strand opposite to that which encodes *proC* in *M. smithii* and is completely contained within the *proC* sequence. It was not considered a *bona fide* gene in a previous publication (12). The RNY rule therefore seems to provide additional support for the conclusion that ORF-D is not a polypeptide encoding gene although this could be an erroneous interpretation of the RNY data if ORF-D is, in fact, an overlapping gene. In contrast, there is convincing evidence that the ORF-*PurE*$_t$ sequence is a polypeptide encoding gene. The polypeptide has been identified by its synthesis in minicells of *E. coli*. An in-frame deletion within ORF-*PurE*$_t$ reduces the size of the encoded polypeptide when synthesized in *E. coli* and also inactivates the ability of the polypeptide to complement mutations in *purE* of *E. coli* (13). The non-conformity of ORF-*PurE*$_t$ with the RNY rule is particularly surprising in that the *purE* complementing gene from a different methanogen, *M. smithii* (ORF-*PurE*$_s$, Table 3) does follow the RNY rule and these two methanogen-derived genes are clearly evolved from a common ancestor. The two DNA sequences are 53% homologous and the encoded polypeptides are 74% homologous if conservative amino-acid substitutions are considered to maintain polypeptide homology (13). It appears therefore that during evolution the divergence which has produced a mesophilic *M. smithii* having a genome with 30.6% G+C and a thermophilic *M. thermoautotrophicum* a genome containing 49.7% G+C has permitted the *M. smithii* but not the *M.* having *thermoautotrophicum purE* gene to maintain the preferential usage of RNY codons.

d) *Codon usage*. It is well established that codon usage is not random; there is a direct correlation in the choice between synonymous codons and the availability of isoaccepting tRNAs (24, 25). Table 4 is a comparison of the codons used by *M. smithii*, *M. thermoautotrophicum*, *M. voltae* and *M. vannielii* with codons used by *E. coli* and *S. cerevisiae*.

Table 3. FREQUENCY OF OCCURENCE OF RNY[*] CODONS IN METHANOGEN OPEN READING FRAMES (ORFs).

Methanogen	ORF[†]	Number of Codons[‡]	Frame[ξ]						Reference containing DNA sequence
			0		1		2		
			RNY	Stop	RNY	Stop	RNY	Stop	
M. smithii	*purE*$_s$	339	120	0	39	33	85	37	(12)
M. thermoauto-trophicum	*purE*$_t$	334	89	0	39	36	94	23	(13)
M. smithii	B_s	418	155	0	58	33	95	52	(12)
M. smithii	IS	401	98	0	91	32	94	47	(12)
M. smithii	*proC*	251	84	0	37	23	58	25	(12)
M. smithii	D	171	40	0	21	13	56	10	(12)
M. smithii	E	237	77	0	41	21	59	31	Unpublished
M. vannielii	1	502	141	0	85	36	121	52	(11)
M. vannielii	3	76	25	0	9	5	23	9	(11)
M. vannielii	*hisA*	238	81	0	26	18	58	27	(11)
M. voltae	*hisA*	242	83	0	25	28	55	20	(11)

[*] RNY codons defined by Shepherd (22, 23); R=purine, Y=pyrimidine, N=purine or pyrimidine.

[†] ORF designations are given in the cited references. The genetic loci indicate that mutations in these genes of *E. coli* are complemented by the the cloned methanogen gene.

[‡] Number of amino-acid encoding codons.

[ξ] Frames 0, 1 and 2 begin with the A, U and G of the AUG initiation codon, respectively. The number of termination codons, UAA, UAG and UGA in each reading frame is listed under 'Stop'.

TABLE 4. CODON USAGE IN E. COLI*, S. CEREVISIAE AND FOUR METHANOGENS†.

	E. coli		S. cere-visiae		M. smithii		M. thermo-autotrophicum		M. voltae		M. van-nielii	
Residue and Codon	No.	% synonym	No.	% synonym	No.	% synonym	No.	% synonym	No.	% synonym	No.	% synonym
Ala GCA	179	22	13	4	39	46	15	33	13	54	30	77
GCC	179	22	78	23	10	12	21	46	–	0	3	8
GCG	236	28	2	>1	–	0	1	2	1	4	1	2
GCU	238	28	249	73	36	42	9	29	10	12	5	13
‡Total	832	10.5	342	10.9	85	5.1	46	8.7	24	4.7	39	5.3
Arg AGA	3	>1	113	88	36	68	10	32	10	62	8	35
AGG	3	>1	4	3	4	7	15	48	2	13	8	35
CGA	14	3	–	0	2	4	–	0	1	6	–	0
CGC	156	33	1	>1	3	6	1	3	–	0	1	4
CGG	17	4	–	0	–	0	3	10	–	0	1	4
CGU	280	59	10	8	8	15	2	7	3	19	5	22
Total	473	6.0	128	4.1	53	3.2	31	5.9	16	3.1	23	3.1
Asn AAC	210	75	105	85	29	25	16	84	6	13	12	34
AAU	69	25	18	15	89	75	3	16	40	87	23	66
Total	279	3.5	123	3.9	118	7.2	19	3.6	46	9.1	35	4.7
Asp GAC	259	55	103	58	36	37	11	35	13	32	10	22
GAU	209	45	75	42	60	63	20	65	28	68	36	78
Total	468	5.9	178	5.6	96	5.7	31	5.9	41	8.1	46	6.2
Cys UGC	51	64	1	6	12	45	6	75	2	29	4	50
UGU	29	36	16	94	15	55	2	25	5	71	4	50
Total	80	1.0	17	0.5	27	1.6	8	1.5	7	1.3	8	1.1
Gln CAA	78	23	82	91	38	79	–	0	15	88	12	75
CAG	256	77	8	9	10	21	4	100	2	12	4	25
Total	334	4.2	90	2.8	48	3.0	4	0.8	17	3.3	16	2.1
Glu GAA	454	75	166	94	103	89	17	43	26	72	53	96
GAG	148	25	11	6	13	11	23	57	10	28	2	4
Total	602	7.6	177	5.6	116	7.0	40	7.6	36	7.1	55	7.4
Gly GGA	24	4	3	1	59	63	11	30	7	18	32	52
GCC	243	42	7	3	7	8	6	16	7	18	4	6
GGG	34	6	4	2	7	8	10	27	4	10	7	13
GGU	280	48	238	94	20	21	10	27	21	54	18	29
Total	581	7.4	252	8.0	93	5.7	37	7.0	39	7.7	61	8.2

TABLE 4. cont

	E. coli		S. cerevisiae		M. smithii		M. thermo-autotrophicum		M. voltae		M. vannielii	
His CAC	98	69	56	75	11	29	3	37	3	60	8	53
CAU	45	31	19	25	27	71	5	63	2	40	7	47
Total	143	1.8	75	2.4	38	2.3	8	1.5	5	1.0	15	2.0
Ilu AUA	4	>1	3	2	71	40	37	82	27	45	25	34
AUC	321	67	83	47	23	13	5	11	4	7	10	13
AUU	154	32	90	51	84	47	3	7	29	48	40	53
Total	479	6.1	176	5.6	178	10.9	45	8.5	60	11.8	75	10.1
Leu CUA	14	2	18	7	14	10	3	6	4	9	6	8
CUC	67	9	4	1	7	5	23	50	1	2	4	5
CUG	505	67	6	2	13	9	10	22	1	2	1	4
CUU	60	8	6	2	36	26	9	19	4	9	27	37
UUA	40	5	31	13	52	37	1	2	29	64	29	40
UUG	64	9	188	74	17	13	–	0	6	13	6	8
Total	750	9.5	253	8.1	139	8.5	46	8.7	45	8.9	73	9.8
Lys AAA	331	73	62	25	152	94	8	28	47	87	63	87
AAG	123	27	185	75	10	6	21	72	7	13	9	13
Total	454	5.7	247	7.9	162	9.9	29	5.5	54	10.7	72	9.7
Met AUG	207	100	55	100	27	100	20	100	16	100	19	100
Total	207	2.6	55	1.7	27	1.6	20	3.8	16	3.1	19	2.6
Phe UUC	167	66	80	77	16	29	10	67	2	83	21	100
UUU	84	34	24	23	39	71	5	33	10	17	–	0
Total	251	3.2	104	3.3	55	3.3	15	2.8	12	2.4	21	2.8
Pro CCA	45	14	95	75	24	38	3	11	3	22	9	41
CCC	20	6	4	3	6	10	14	52	4	28	6	27
CCG	69	69	11	8	9	14	4	15	1	7	–	0
CCU	11	11	21	16	24	38	6	22	6	43	7	32
Total	316	4.0	131	4.2	63	3.8	27	5.1	14	2.8	22	3.0
Ser AGC	86	21	2	1	14	15	9	21	2	14	2	5
AGU	27	7	8	3	22	23	4	10	3	20	9	23
UCA	23	6	12	5	28	29	14	33	3	20	20	51
UCC	118	29	96	40	10	10	8	19	–	0	1	2.5
UCG	43	11	1	>1	–	0	2	5	–	0	1	2.5
UCU	106	26	119	50	22	23	5	12	7	46	6	15
Total	403	5.1	238	7.6	96	5.9	42	8.0	15	3.0	39	5.3

TABLE 4. cont.

	E. coli		S. cerevisiae		M. smithii		M. thermoautotrophicum		M. voltae		M. vannielii	
Thr ACA	25	6	14	7	40	43	3	20	11	50	15	42
ACC	205	54	76	41	16	17	7	47	3	14	3	8
ACG	44	11	2	1	3	3	2	13	1	4	7	19
ACU	105	28	95	51	33	37	3	20	7	32	11	31
Total	379	4.8	187	5.9	92	5.7	15	2.8	22	4.3	36	4.9
Trp UGG	30	100	25	100	3	100	2	100	5	100	6	100
Total	30	0.4	25	0.8	3	0.2	2	0.4	5	1.0	6	0.8
Tyr UAC	119	60	71	81	20	34	4	50	7	39	8	33
UAU	79	40	17	19	38	66	4	50	11	61	16	67
Total	198	2.5	88	2.8	58	3.4	8	1.5	18	3.5	24	3.2
Vol GUA	138	22	6	2	42	42	11	20	18	41	14	25
GUC	86	14	108	43	6	6	8	14	2	4	1	2
GUG	154	24	9	3	2	2	16	29	8	18	5	9
GUU	252	40	129	54	49	50	21	37	16	36	35	64
Total	630	8.0	252	8.0	99	6.1	56	10.6	44	8.7	55	7.4
% G+C of genome	51		36		31		50		31		31	

* Codon usages for approximately 50 *E. coli* genes and 15 *S. cerevisiae* genes, originally tabulated by Ikemura and Ozeki (25), have been used in construction of Table 4.

† The methanogen sequences are given in publications (11-13).

‡ The total number of codons encoding the same amino-acid. This number is given as a percentage of all the amino acids under the % synonym heading.

The genome of *E. coli* contains 51% G+C, almost the same G+C content as in the genome of *M. thermoautotrophicum*. Similarly the genome of *S. cerevisiae* contains 36% G+C which is close to the 31% G+C content of the genomes of *M. smithii*, *M. voltae* and *M. vannielii*. Codon preferences of the methanogens seem to be dominated by the relative availability of A/T and G/C base pairs. As examples, the lysine codon AAA and asparagine codon AAU are preferentially used by *M. smithii*, *M. voltae*, and *M. vannielli*, species in whose genomes G/C base pairs occur relatively infrequently, whereas the lysine codon AAG and asparagine codon AAC are preferentially used by *M. thermoautotrophicum*, a species in which G/C pairs constitute a much higher percentage of the total genome. This simple correlation does not hold so consistently for *E. coli* and *S. cerevisiae*. *E. coli* (51% G+C) employs AAA more often than AAG whereas

S. cerevisiae (36%) preferentially uses AAG and AAC rather than AAA and AAU. The somewhat unpredictable codon usages of *E. coli* and *S. cerevisiae* can be explained once the relative availabilities of different tRNAs in these species is considered. Codons AAA and AAG are recognized by a single $tRNA_{LYS}$ in *E. coli* whereas *S. cerevisiae* has two lysine accepting tRNAs both of which recognize AAG but only one of which functions with AAA codons (25). Unfortunately considerations of this type cannot be applied in evaluating the codon usages in methanogens as the number and relative amounts of isoaccepting tRNAS have not been fully determined. It will be interesting, once these experiments are completed, to see how well the actual amounts of isoaccepting tRNAs agree with predictions for these values which can be made from Table 4. In addition to conforming directly with the need to accommodate different G+C contents, codons preferentially used by methanogens are often codons which are almost never used by *E. coli* e.g. AUA, AGA and AGG. In this respect codon usages by methanogens are more similar to codons usages of the eucaryote, *S. cerevisiae*. Very infrequent usage of codons containing the dinucleotide CG, which is almost always underpresented in eucaryotic genomes (26), is evident in the lists of codons used by *S. cerevisiae* and by all four of the methanogens. Selectivity in codons employed is not limited to choices between different codons designating use of the same amino-acid but also influences the net usage of different amino-acids. The polypeptide products of the methanogen genes analyzed in Table 4 contain relatively more isoleucine and lysine residues and less alanine residues than are found in the *E. coli* and *S. cerevisiae* gene products.

Promoter structure and expression of methanogen genes in E. coli. It was evident from the first publication describing cloning and expression of methanogen genes (27) that methanogen derived DNAs must contain sequences which can function as promoters in *E. coli*. Analysis of the actual DNA sequences that are now available confirms this expectation (11-13). The genomes of *M. smithii*, *M. voltae* and *M. vannielii* are composed of almost 70% A+T base pairs and the intergenic regions contain sequences which approach 90% A+T. Sequences which function as promoters in *E. coli* are known to be very A/T rich. Many acceptable versions of the consensus -35(TTGACA) and -10(TATAAT) *E. coli* promoter squences can be found positioned in locations which should facilitate transcription of adjacent cloned methanogen genes in *E. coli*. Availability of ribosome binding sequences (Table 2) apparently ensures that if a methanogen gene is transcribed in *E. coli*, the transcripts can be translated.

The structure of *bona fide* methanogen promoters remains to be determined. A comparison of DNA sequences which precede both genes from methanogenic and halophilic archaebacteria lead to the proposal that 5'GAANTTTCA and 5'TTTTAATATAAA might be consensus archaebacterial promoter sequences (12, 13). An intriguing correlation is that these sequences are contained within sequences previously identified as promoters in *Drosophila* (28). It has also been shown that the *Drosophila* promoters are recognized in cells of the yeast *S. cerevisiae*. As the DNA-dependent RNA-polymerases of archaebacteria are apparently structually related to RNA polymerases of yeast (29) the similarity of archaebacterial and *Drosophila* promoters may be more than coincidental. Development and use of *in vitro* transcription systems using purified DNA-dependent RNA-polymerases from methanogens is in progress and should help confirm or negate the proposed archaebacterial promoter sequence.

CONCLUSIONS

We now have the complete nucleotide sequence for more than ten different methanogen genes including genes from four different methanogenic species (Table 1). These genes direct the synthesis of enzymes when cloned in *E. coli*. The overall structures and organizations of methanogen genes, as described in this report, are very similar in most respects to the structures and organizations well established for classical prokaryotes such as *E. coli* and *B. subtilis*. This result is very encouraging in that it predicts that sophisticated genetic engineering procedures, developed for use with these thoroughly studied prokaryotes, ought to be directly applicable to methanogens. We and other research groups (16) have therefore already begun cloning genes which encoded enzymes that catalyse biochemical steps in methanogenesis. Initial results of these studies demonstrate that it is possible to clone such genes in *E. coli*. The resulting availability of "methane" genes now offers the opportunity of genetically manipulating methanogenesis using *in vitro* genetic engineering techniques. Two attractive goals of such endeavors would be increasing the range of microbial species which can produce methane and be expanding the range of substrates and environments which can support methanogensis. The feasibility of such projects and practical approaches to reaching these goals are the major themes of experiments we now have in progress.

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THE START OF NITRIFIER GENETICS

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ABSTRACT

Two bacterial groups, ammonia-oxidizers and nitrite-oxidizers, acting together, are the primary microbes involved in the conversion of ammonia to nitrate. We have begun a study of the genetic structure and organization of Nitrobacter hamburgensis strain X14, a nitrite-oxidizer. The gene which codes for β-isopropylmalate dehydrogenase in this organism has been successfully cloned. We are sequencing the gene and its flanking regions in order to learn the DNA sequences in Nitrobacter which are used for transcription and translation of genes. This information will be used to construct plasmid vectors with which to introduce genes into N. hamburgensis X14.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

INTRODUCTION

Nitrification is a desirable process in wastewater treatment and is brought about largely by the concerted action of two groups of autotrophic bacteria which together comprise the bacterial family Nitrobacteraceae. One group, designated by the prefix Nitroso, contains numerous members all of which can oxidize ammonia to nitrite; they are referred to as ammonia-oxidizers. The second group, designated by the prefix Nitro, contains various members all of which can oxidize nitrite to nitrate; they are referred to as nitrite-oxidizers. The term nitrifiers is used when referring to both groups collectively. Despite their significant contribution to wastewater treatment and to the global nitrogen cycle, virtually nothing is known of the genetics of nitrifiers. This laboratory has begun a program designed to discover the genetic structure and organization of Nitrobacter hamburgensis strain X14 (1), a nitrite-oxidizing bacterium. The results will provide the type of information eventually required to apply genetic engineering to nitrifiers in order to produce strains which will increase the rate of nitrification in wastewater treatment facilities. The approach which we think will be ultimately most useful in increasing the nitrification rate is the introduction of genetic information into nitrifiers that will allow them to grow faster in their unique biological niches such as wastewater treatment facilities. Our immediate goal is the introduction of genes into N. hamburgensis strain X14 which will increase its growth rate in autotrophic medium. This medium is as comparably modest in nutritional value as wastewater. Which genetic information do we think will produce a more rapidly growing N. hamburgensis strain X14? Specifically, we will introduce DNA derived from other nitrite oxidizing members of the Nitrobacteraceae family especially those which occupy global ecological niches other than waste water treatment plants. Another potentially useful DNA donor would be Rhodopseudomonas palustris (2) which has been shown recently (3) to be closely related phylogenetically to Nitrobacter.

N. hamburgensis[1] is a Gram-negative autotrophic bacterium which can obtain carbon and nitrogen for synthesis of protoplasm from atmospheric CO_2 and nitrite. Its only other requirements are water, oxygen, phosphate and other minerals. From this simple medium it can synthesize all of the necessary building blocks, e.g., amino acids, purines, pyrimidines, vitamins, etc. It is assumed that the biosynthesis of these molecules involves the same pathways used by the familiar heterotroph Escherichia coli but this point has never been investigated. In fact the actual demonstration of the existence of any familiar enzymatic reactions in nitrite-oxidizers is largely confined to those involved in energy yielding processes. Ribulose-1,5-bisphosphate carboxylase (4) and nitrite oxidoreductase (5) have been demonstrated in cell free extracts. The former is a key enzyme for the fixation of CO_2 in nitrifiers via the Calvin cycle. The latter is the enzyme complex responsible for nitrite oxidation; it has been purified and characterized recently (6). We chose to use Nitrobacter hamburgensis strain X14, henceforth simply strain X14, in our studies because it grew relatively rapidly, its nitrite-oxidizing activity was unusually high (1) and it, as well as the related strain Y (1), were the only nitrite-oxidizers which contained plasmids (7).

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[1] At present two strains of N. hamburgensis, designated X14 and Y, have been identified(1).

EXPERIMENTAL

The purpose of our first experiments was to learn if an antibiotic resistance plasmid could be introduced into strain X14. We chose to use plasmids called broad host range plasmids since they have been readily introduced and replicated in numerous other Gram-negative organisms (8). They contain antibiotic resistance genes which would allow detection of their entry and replication in strain X14. Although there were no reports of the introduction of broad host range plasmids into nitrifiers the existence of plasmids in N. hamburgensis strains X14 and Y indicated that some plasmids can be maintained in nitrite-oxidizers. Introduction of plasmids into new bacterial hosts can be by means of either conjugation or transformation (8,9,10). The antibiotic resistance genes are especially useful because if the plasmid is introduced and maintained the host is then resistant to an antibiotic to which it was formerly susceptible. Our first experiments were designed to introduce the broad host range antibiotic resistance plasmid RP1(8) and several derivatives of RP1 (11,12) into strain X14 by means of conjugation and transformation. By either means success would have been evident from the growth of strain X14 on antibiotic supplemented agar plates. Despite the use of a variety of plasmids and various published experimental protocols for conjugation (13) and transformation (14,15) we did not recover any antibiotic resistant isolates of strain X14. Two, of several, possible explanations for our results are seen in Table 1.

TABLE 1. POSSIBLE OUTCOMES OF CONJUGATION AND TRANSFORMATION OF STRAIN X14 BY BROAD HOST RANGE PLASMIDS

Outcome	Conclusions
A. Isolation of antibiotic resistant isolates of strain X14.	A.1. The DNA sequences required for transcription and translation of plasmid genes are functional in strain X14.
B. No antibiotic resistant isolates of strain X14 are obtained.	B.1. The DNA sequences required for transcription and translation of plasmid genes are not functional in strain X14. OR B.2. The origin of replication of the plasmid is not functional in strain X14.

We chose to investigate first the possibility that genes in broad host range plasmids are not functionally expressed in strain X14. Functional expression of a plasmid gene, like any bacterial gene, requires the presence of: DNA sequences at the 5' end of the gene which permit transcription of the gene into messenger RNA (mRNA) by the cellular RNA polymerase; Shine-Dalgarno sequences (16) in the mRNA which allow ribosomes to attach to mRNA; translation termination sequences at the 3' end of the gene and; transcription termination sequences at the 3' end of the gene. In order to learn what sequences strain X14 uses for transcription and translation we would have to isolate a gene from strain X14 and determine its DNA sequence.

The isolation of a gene from strain X14 was achieved as follows. The chromosomal DNA of strain X14 was digested partially (10) with the restriction enzyme Sau3A and the resultant 5 kilobase (kb) fragments were isolated from low melting temperature agarose (10). The fragments were introduced into the BamHI site in the plasmid vector pMK2004 (Figure 1; reference 17); this produced a mixture of recombinant plasmids.

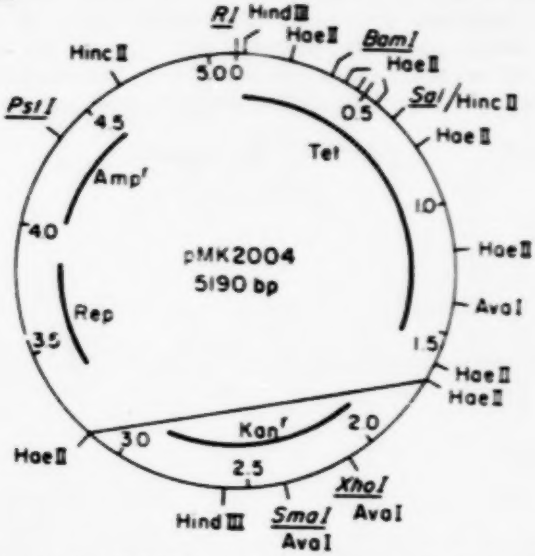

Figure 1. Restriction map of pMK2004. Coordinates are in kilobase pairs. Tet, tetracycline resistance gene, Kan^r, kanamycin resistance gene, Rep, origin of replication, Amp^r, ampicillin resistance gene.

This mixture was used to transform E. coli C600 (10), a strain which has two relevant mutations in its chromosome. One is a mutation in the gene leuB which is a gene that codes for β-isopropylmalate dehydrogenase, one of three enzymes required for leucine biosynthesis. Because of this mutation E. coli C600 cannot grow unless it is provided with leucine in the medium and is said to be leucine-dependent. A second mutation, in the thrB gene of E. coli C600, causes it to be threonine-dependent as well as leucine-dependent. After the recombinant plasmids were introduced into E. coli C600 by the process of transformation, we were able to recover twelve clones of this strain which were no longer leucine-dependent. In a separate transformation we recovered three clones of E. coli C600 which were no longer threonine-dependent. The successful recovery of both leucine and threonine independent clones indicated strongly that genes of strain X14 were functionally expressed in E. coli. Let us focus only on the recombinant plasmids in the leucine independent transformants. We used them for a series of experiments designed to obtain the smallest piece of strain X14 DNA which could express β-isopropylmalate dehydrogenase. This DNA would then be sequenced in order to learn which sequences are used for transcription and translation in strain X14. We focused on seven of the recombinant plasmids since the other five were unstable. Three of the seven contained an approximately 12 kb fragment of strain X14 derived chromosomal DNA; the other four contained an approximately 6 kb fragment. In all probability the three larger plasmids were reisolates of the same plasmid; the same is probably true of the four smaller plasmids. One of the larger plasmids, pNBH6, and one of the smaller plasmids, pNBH3, were digested with several restriction enzymes. The resultant data were used to draw the restriction maps of their respective fragments (Figure 2).

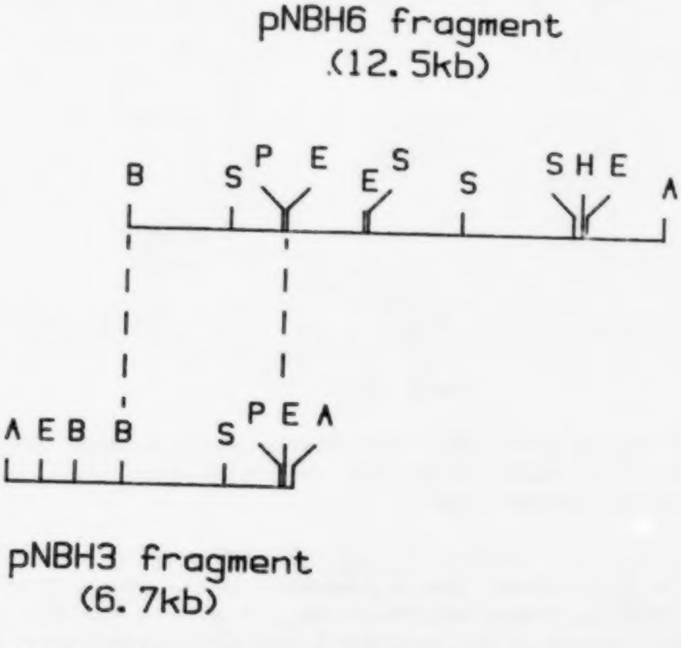

Figure 2. Partial restriction map of the strain X14-derived fragments within recombinant plasmids pNBH3 and pNBH6 which confer leucine independence on E. coli C600. A, Sau3A, B, BamHI, E, EcoRI, H, HindIII, P, PstI, S, SalI, kb, kilobases.

It was evident that the leuB gene of strain X14 must be located in the common BamHI -EcoRI fragment. Upon subcloning this fragment into pMK2004 the resultant recombinant plasmid, pNBH601 (Figure 3), was found to confer leucine independence on E. coli C600.

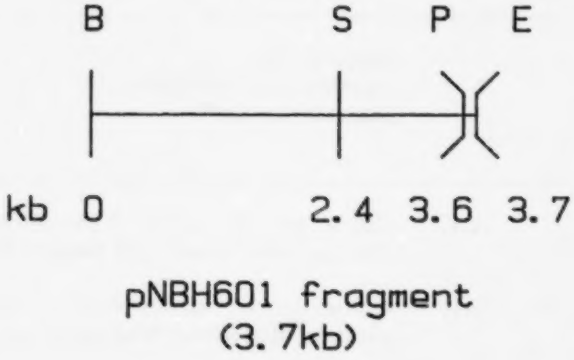

Figure 3. Partial restriction map of the strain X14-derived fragment in pNBH601 which confers leucine independence on E. coli C600. B, BamHI, E, EcoRI, S, SalI, P, PstI, kb, kilobases.

The additional subcloning of the BamHI - SalI fragment of pNBH601 into pMK2004 produced the plasmid pNBH602 (Figure 4) which also conferred leucine independence on E. coli C600.

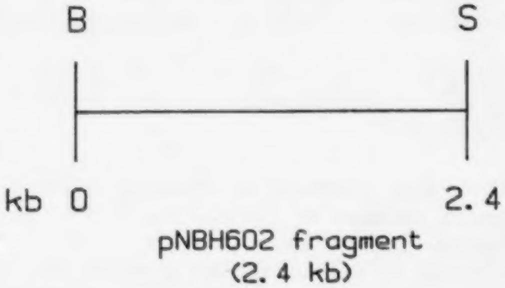

Figure 4. Partial restriction map of the strain X14-derived fragment in pNBH602 which confers leucine independence on E. coli C600. B, BamHI, S, SalI, kb, kilobases.

We are now sequencing this fragment in order to learn a number of things. The most important piece of information we will derive from the DNA sequence is whether strain X14 uses the same transcription and translation sequences that E. coli uses. Once we have determined whether the transcription and translation sequences are the same or different we will construct a shuttle plasmid vector. The steps involved in the construction will depend on whether the transcription and translation sequences of strain X14 and E. coli are identical or different (Table 2).

TABLE 2. CONSTRUCTION OF A SHUTTLE PLASMID VECTOR

Transcription and translation sequences of E. coli and strain X14		Steps in the construction of shuttle plasmid vector*
A. Identical	A.1.	Clone ori[§] of strain X14 chromosome or plasmid into broad host range plasmid
B. Different	B.1.	Clone ori of strain X14 chromosome or plasmid into broad host range plasmid
	B.2.	Use in vitro recombinant DNA techniques to position strain X14 transcription and translation sequences at 5' and 3' end of an antibiotic resistance gene in the plasmid

*Shuttle plasmid vector can be broadly defined as a plasmid capable of replication in two microbial hosts which are taxonomically distinct. One example is the plasmid pHV14 (18) which can replicate in E. coli and Bacillus subtilis.

[§] The origin of replication (ori) is the DNA sequence present on all replicons (chromosome, plasmid, phage) which is necessary for the replicon's replication.

At the successful conclusion of the experiments outlined in Table 2 we will have available a shuttle plasmid vector capable of introducing genes into strain X14. We will introduce into that plasmid chromosomal DNA from other organisms. The organisms chosen will be those that we believe contain genes which when expressed in strain X14 will increase its growth rate in autotrophic medium. As noted earlier two sources of chromosomal DNA which seem most appropriate are those of other nitrite-oxidizers and R. palustris. The resultant plasmid will be introduced into strain X14 and standard microbial genetic procedures used to isolate faster growing antibiotic resistant isolates. The genetic engineering of ammonia-oxidizers would presumably follow similar lines in order to produce a strain capable of faster growth in autotrophic medium. The introduction of faster growing nitrifiers into wastewater treatment facilities would have two beneficial effects. First, the overall rate of nitrification would be increased. Second, the faster growing strains of nitrifiers would be able to outgrow any competing nitrifiers which enter the wastewater treatment facility.

RESULTS AND/OR BENEFITS EXPECTED

Nitrification of wastewater is a useful process since it reduces the ultimate BOD in the effluent to an acceptable level. The slow rate of nitrification adds to the construction and operating cost of wastewater treatment facilities. The reliability of nitrification is not consistent because periodic changes (seasonal, diurnal) in nitrifying activity result in incomplete nitrification. Introduction of a more rapidly growing strains of nitrifiers would decrease operating costs (less retention time), decrease construction costs (eliminate the need for long-term aeration) and increase the reliability of nitrification (more ecologically stable system).

Although nitrification is one limb of the overall nitrogen cycle that occurs throughout the biosphere, the genetics, microbial physiology and biochemistry of nitrification are poorly understood. Increased knowledge about nitrification may permit applications in other waste treatment problems and in areas such as agriculture, which depend on operation of the cycle and the use of inorganic nitrogen as fertilizer.

CONCLUSION

A gene has been isolated from strain X14, a nitrite-oxidizer. This will permit us to determine the DNA sequences used by strain X14 for transcription and translation of their genes. This information will be used to construct a shuttle plasmid which contains an antibiotic resistance gene which should be functionally expressed in strain X14. The shuttle plasmid will serve as the vector for introducing bacterial DNA into strain X14 in order to increase the growth rate of the organism in autotrophic medium.

ACKNOWLEDGEMENT

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REMOVAL OF PHOSPHATE AND OTHER SMALL MOLECULES FROM WASTE STREAMS WITH BINDING PROTEINS IN CYCLING COLUMN ADSORBERS

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This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

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ABSTRACT

This report describes the use of a special class of stable, reversibly denaturable proteins with high substrate affinity and specificity in cycling column adsorbers designed for the removal of specific small molecules from waste streams.

The class of proteins termed "binding proteins" resides in the space between the inner and outer membranes (periplasmic space) of the gram-negative bacteria. These proteins are involved in transporting nutrients across the plasma membrane. They bind their respective nutrients with high specificity and affinity. In addition, they are resistant to proteases and heat. Further, these proteins bind their respective substrates through a broad range of pH values and ionic strengths. These special properties make this class of proteins especially interesting to consider for use in cycling column adsorbers for removal of specific small molecules from waste streams, or other aqueous environments.

We have attached the phosphate-binding protein from *E. coli* to cyanogen bromide activated Sepharose 4B (Pharmacia). Radiolabeled phosphate (^{33}-Pi) was used to monitor the performance of the resin-bound protein. We demonstated that the solid phase phosphate-binding protein efficiently removed phosphate from a feed stream. The bound phosphate could be released from the binding protein by reversibly denaturing the phosphate-binding protein. Upon renaturing, the resin-bound phosphate-binding protein could be subjected to continuous cycles of loading, and unloading. Batch experiments were also carried out with a glutamate-aspartate binding protein. It behaved in a similar manner.

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To facilitate the production of the phosphate-binding protein, a bacterial strain containing a plasmid bearing the gene that encodes this protein was used for the large-scale purification of protein. To increase the efficiency of the purification procedure, a mutant strain that secretes its periplasmic proteins directly into the medium is being designed for high level production of the phosphate-binding protein.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

INTRODUCTION

The unique properties of the class of bacterial nutrient transport proteins termed "binding proteins" made them worth considering as candidates to use in cycling column adsorbers for the removal of specific small molecules from waste streams. Proteins offer the possibility of high affinity binding and a high degree of substrate specificity. For example, they can easily differentiate chemical isomers. If a simple procedure is designed for the removal of the bound substrate and renaturation of the active protein, it should be possible to design affinity columns for the removal of many different molecules from solution. We have chosen the phosphate-binding protein of E. coli as a model system, since it has been cloned and can be produced in reasonable quantity. The experiments described below demonstrate that solid phase proteins with high affinity, high specificity and high stability can be used in cycling column adsorbers to scrub specific small molecules from solution.

EXPERIMENTAL PROCEDURES

MATERIALS AND METHODS

Bacterial Strains. E. coli strain AN1685 transformed with plasmid pAN92 was supplied by Dr. H. Rosenberg.

Measurement of Phosphate Binding to the Phosphate-binding Protein. The binding of phosphate to the binding protein was determined by equilibrium dialysis (1) or by a simple membrane filter assay (2).

Purification of Phosphate-Binding Protein. Phosphate-binding protein (3) was purified from the periplasmic protein fraction of an E. coli strain transformed with a plasmid bearing the gene that encodes the phosphate-binding protein. The overproduction was achieved by growing this organism on a phosphate deficient medium. The phosphate-binding protein used for the described experiments was purified from osmotic shock fluid from the overproducer strain by gel filtration through a Biogel P-100 column.

Osmotic Shock Procedure. The periplasmic protein fraction was prepared from a 10 liter culture by a standard osmotic shock procedure (4) or a recently developed chloroform extraction procedure (5).

Analytical Gel Electrophoresis Procedures. Standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (6) was used to analyze the protein composition for fractions of interest.

Coupling of Protein to the Solid Phase Support -- Phosphate-binding protein was coupled to cyanogen bromide activated (7) solid phase support, Sepharose 4B. The protein loaded gel (2 mg protein/ml resin bed vol.) was packed into a water jacketed column (1.6 x 5 cm).

Analytical Apparatus. The column feed line was connected to a valve that switched between the solution containing labeled ligand (0.8 uM, ^{33}Pi, 50 uCi/l, in 20 mM Tris-HCl, pH 7.0) and the wash solution (20 mM Tris-HCl, pH 7.0). The column eluate was joined to a stream of scintillation solution and run through a continuous flow scintillation counter (Radiomatic Flo I, Tampa, FL). The feed stream flow rate was 1 ml/min and the scintillation flow 10 ml/min. Data were recorded both as linear counts/time from a printer and log counts/time on a strip chart recorder. A diagram of the apparatus is shown in Figure 1, and a photograph in Figure 2.

Protein Assays. Protein was assayed by the method of Lowry (8).

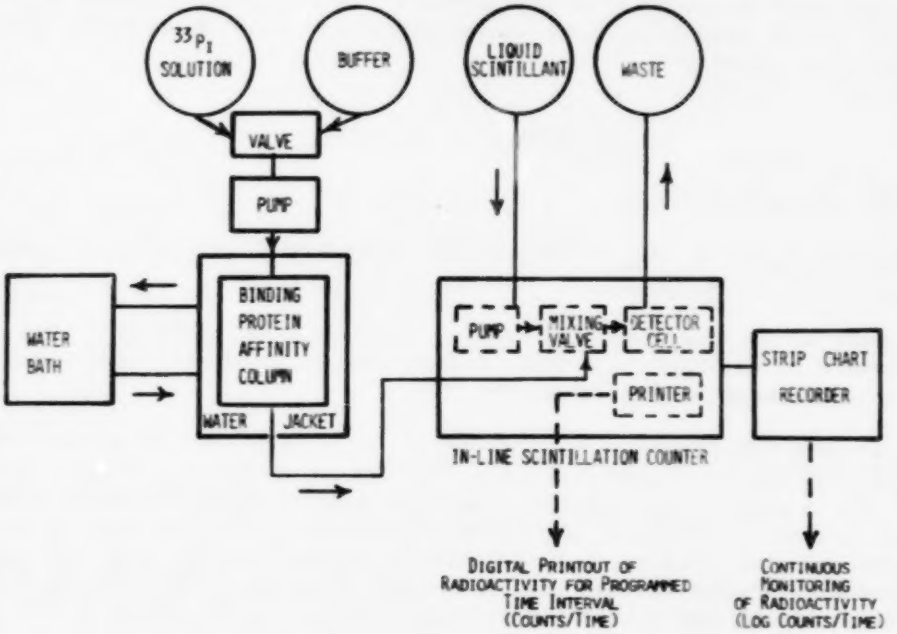
$^{33}P_i$ SOLUTION
BUFFER
LIQUID SCINTILLANT
WASTE
VALVE
PUMP
WATER BATH
BINDING PROTEIN AFFINITY COLUMN
WATER JACKET
PUMP
MIXING VALVE
DETECTOR CELL
PRINTER
STRIP CHART RECORDER
IN-LINE SCINTILLATION COUNTER
DIGITAL PRINTOUT OF RADIOACTIVITY FOR PROGRAMMED TIME INTERVAL (COUNTS/TIME)
CONTINUOUS MONITORING OF RADIOACTIVITY (LOG COUNTS/TIME)

FIGURE 1. DIAGRAM OF THE PHOSPHATE-BINDING PROTEIN REACTOR

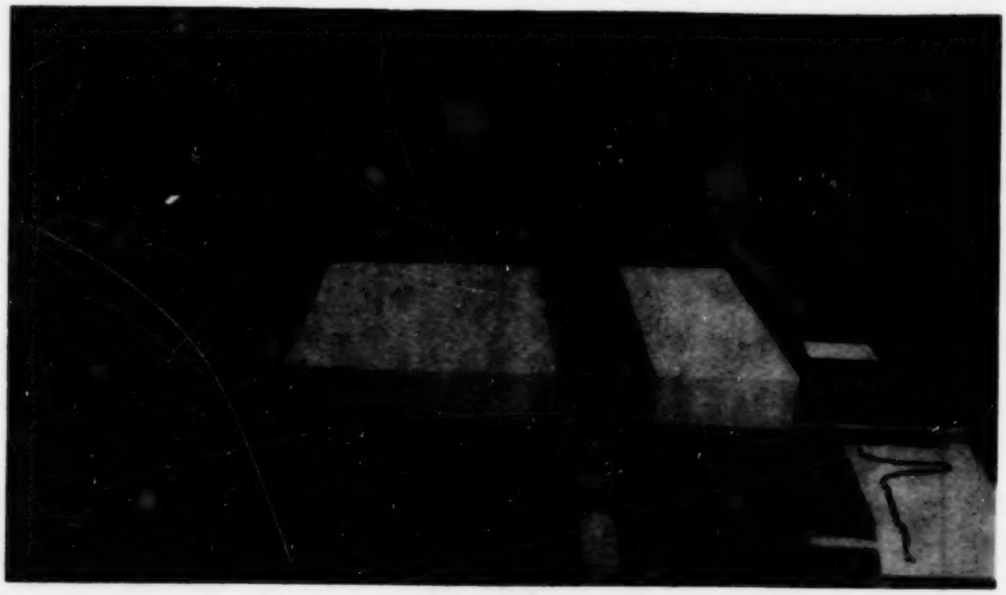

Figure 2. Photograph of the phosphate adsorber and in-line scintillation detector.

RESULTS

The goal of our initial studies was to determine if a cycling adsorber could be constructed with proteins on a solid phase support. For this to be possible, the proteins used needed to have the following properties:

- High affinity and specificity for the solute
- Ability to reversibly denature and release bound solute under convenient and inexpensive conditions that would be useful in waste stream treatment
- Ability to renature after ligand release and undergo many cycles of denaturation/renaturation

The phosphate-binding protein of E. coli was chosen for our initial studies, since it could be produced in quantity, and binding could be monitored with a relatively inexpensive and safe isotope, ^{33}-Pi.

Figure 3 shows the gel filtration purification of the phosphate binding protein from the periplasmic protein fraction of E. coli. A disk gel electrophoretic analysis of the periplasmic protein fraction and purified protein is shown in Figure 4. The single step purification produces highly purified phosphate-binding protein. This is possible since the strain produces such a high level of this protein when grown on low phosphate medium. The phosphate-binding protein comprises between 60-70% of the entire periplasmic protein fraction.

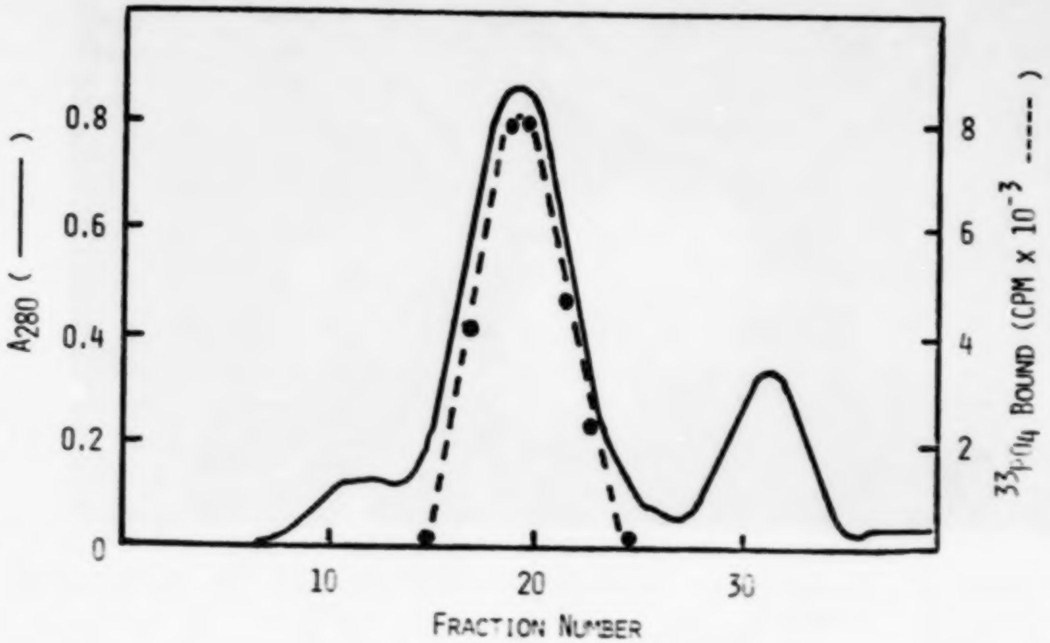

Figure 3. Gel filtration chromatography of osmotic shock fluid.

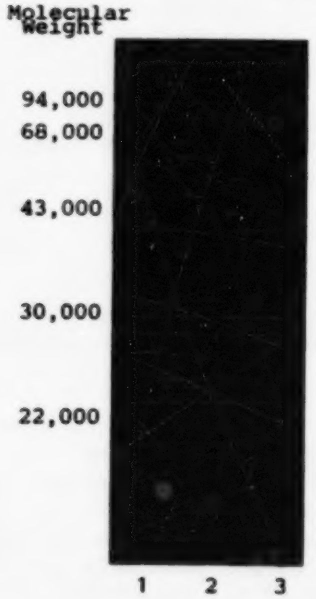
Molecular Weight
94,000
68,000
43,000
30,000
22,000
1
2
3

Figure 4. SDS-PAGE analysis of the purification of the phosphate-binding protein. Lane 1, molecular weight markers: 94,000, phosphorylase B; 68,000 bovine serum albumin; 43,000 ovalbumin; 30,000, carbonic anhydrase; 22,000, soybean trypsin inhibitor. Lane 2, crude shock fluid. Lane 3, purified phosphate-binding protein.

The purified protein was coupled to the solid support resin Sepharose 4B at a density of 2 mg protein/ml resin. The protein conjugated resin was packed into a water jacketed column as described above. A feed stream containing ^{33}-Pi was passed through the column and the outflow was continuously monitored with an in-line scintillation counter. A three-way valve allowed switching between the feed stream and the wash buffer as indicated in each experiment. A schematic representation of the solid-phase protein is presented in Figure 5.

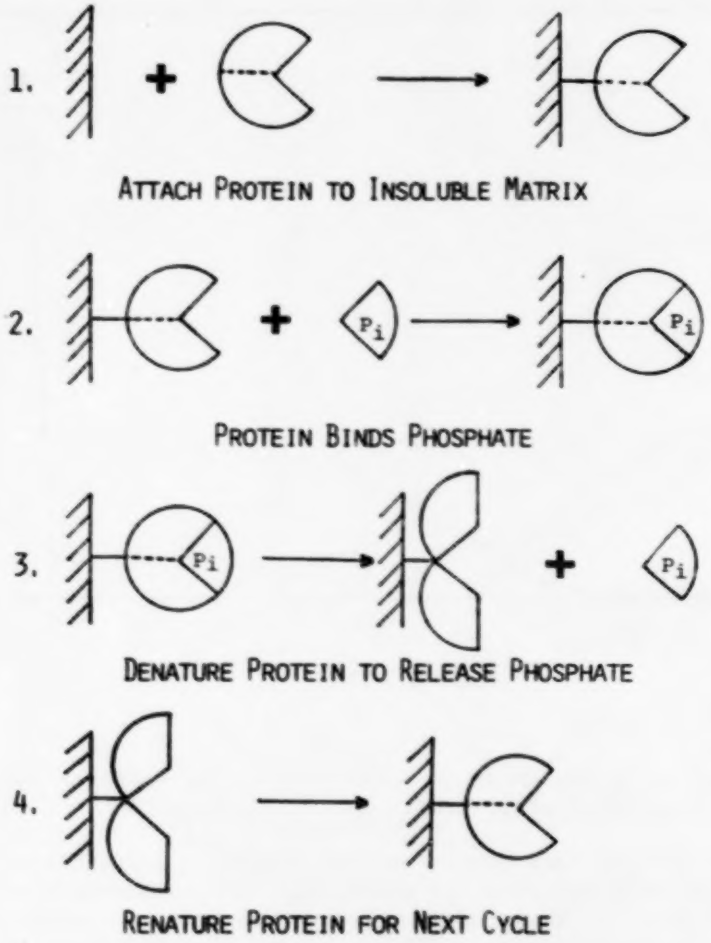
1.
+
ATTACH PROTEIN TO INSOLUBLE MATRIX
2.
+
P_i
P_i
PROTEIN BINDS PHOSPHATE
3.
P_i
+
P_i
DENATURE PROTEIN TO RELEASE PHOSPHATE
4.
RENATURE PROTEIN FOR NEXT CYCLE

Figure 5. Diagram of the attachment of the phosphate-binding protein to the solid phase resin and its reversible binding of phosphate.

A series of experiments were carried out with the phosphate affinity column. Figure 6 shows a run in which the column was loaded to full capacity, then rinsed with rinse buffer, and finally stripped by reversibly denaturing the phosphate-binding protein. From the trace, several things are observed. The column efficiently removed the labeled phosphate from the stream until the ligand sites began to saturate. As the binding capacity of the column was approached, counts appeared in the effluent. If, as in this experiment, the column was allowed to fully saturate before beginning the wash and elution steps, there was a long bleed of isotope from the column. During the steady state rinse, the protein was denatured. It is clear that denaturation of the protein resulted in the release of its bound solute into the rinse stream.

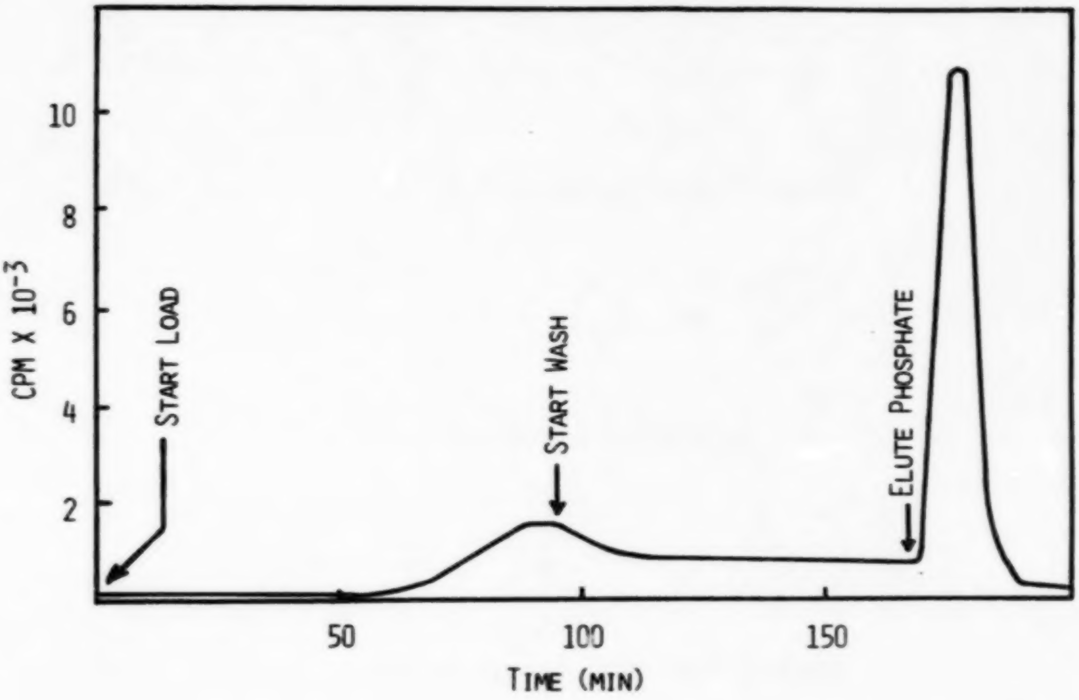

Figure 6. Loading of the phosphate adsorber to full capacity followed by washing and elution of the bound phosphate.

Figure 7 shows another variation of column loading and elution. In this experiment, the valve was rerouted to run the feed stream directly through the scintillation counter. When steady state feed was achieved, the system was washed with buffer, then the feed stream was switched to the column. When counts began to appear in the eluate, the valve was switched to the rinse buffer and the bound phosphate was released by denaturing the protein.

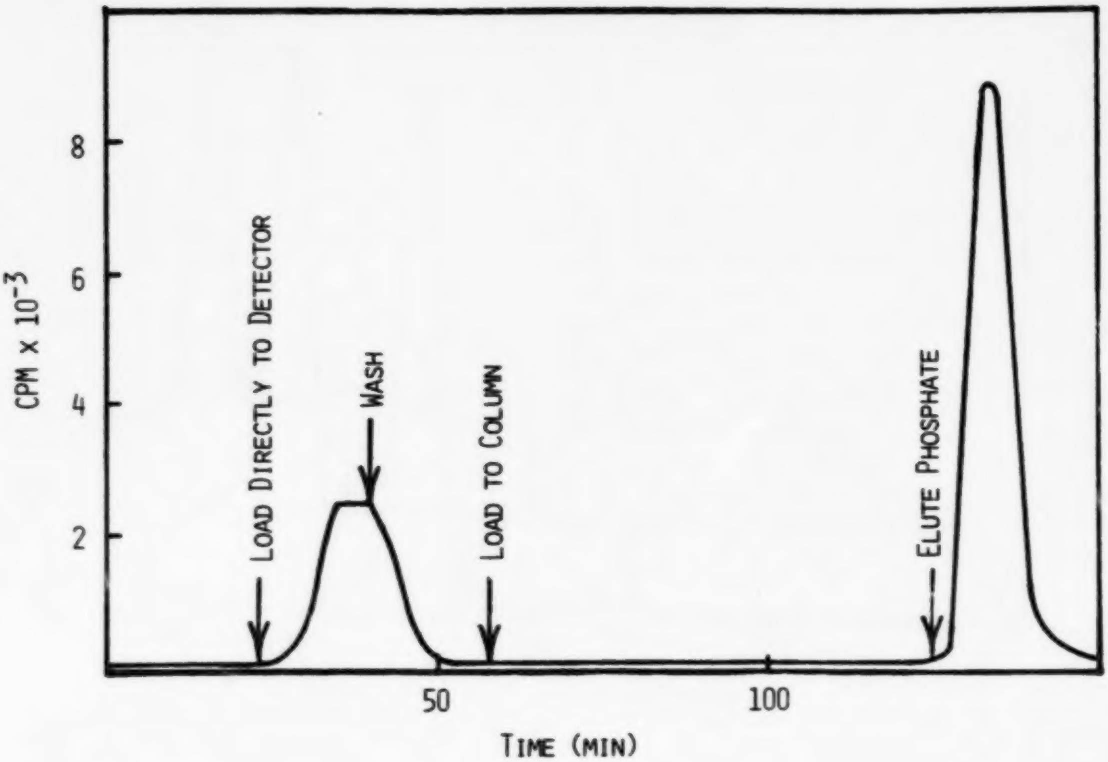

Figure 7. Loading the feed stream directly to the detector system, then through the column, followed by elution of the bound phosphate from the column into the rinse buffer.

The next major question was whether the protein could be renatured and function in subsequent cycles of binding/denaturation/renaturation. Figure 8 shows three consecutive cycles of loading, washing, solute release by denaturation, and renaturation. The immobilized phosphate-binding protein appeared to loose little activity during consecutive cycles. For these runs, the wash was started as soon as the counts began to rise above the background feed level.

These experiments demonstrate that a protein with high affinity and specificity for solute can be attached to solid phase supports and used to scrub the specific solute from solution. Further, the protein is capable of reversible binding through denaturation/renaturation.

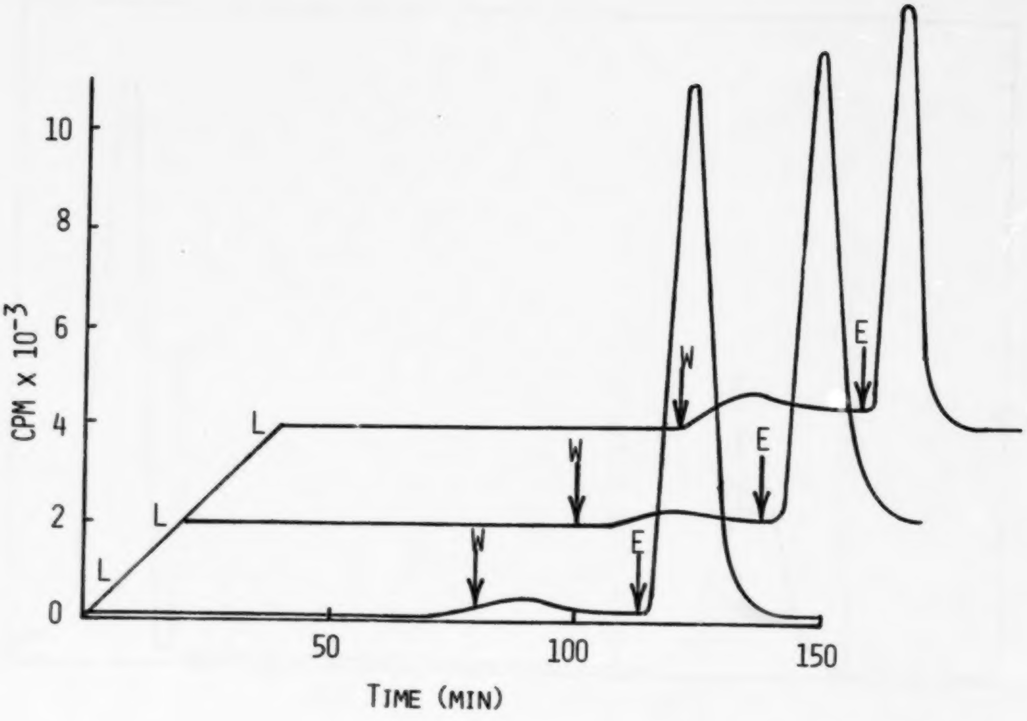

Figure 8. Continuous cycles of phosphate binding/unloading. L indicates the beginning of phosphate loading; W indicates the wash step and E, the elution of the bound phosphate.

The next question is whether large quantities of protein can be produced economically. Two major considerations come into play in designing automated procedures for producing large quantities of the desired protein(s). First, It will be important to engineer strains that produce large quantities of the desired protein(s). Second, it would be most efficient if the overproduced protein(s) could be secreted directly into the medium. If these two criteria can be met, automated, large-scale protein generation procedures can be developed.

The first problem was approached by using a mutant of E. coli that secretes its periplasmic proteins into the medium. Figure 9 shows that this strain appears to also secrete the phosphate-binding protein directly into the medium. Thus, the first problem of achieving direct secretion of the phosphate-binding protein into the medium appears to be solved.

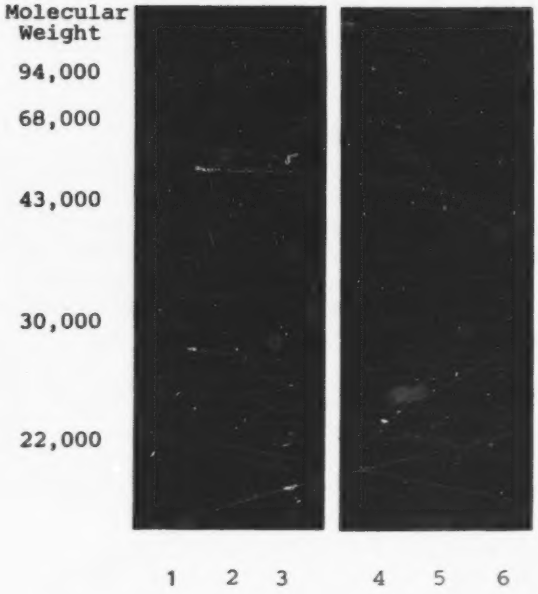

Figure 9. SDS-PAGE analysis of the production and secretion of the phosphate-binding protein by E. coli strain C90. Lane 1, molecular weight markers (see Figure 4). Lane 2, Phosphate-binding protein standard (5 ug). Lane 3, chloroform extracted periplasmic fraction of strain C90 (50 ug). Lane 4 molecular weight markers. Lane 5, phosphate-binding protein standard. Lane 6, concentrated growth medium from strain C90 (50 ug).

The second problem, that of producing strains that generate and secrete very high levels of binding protein into the medium, is presently being pursued. The rationale of the general approach is as follows:

The level of the phosphate-binding protein is controlled by the intracellular level of inorganic phosphate. The phosphate level in turn is controlled by the level of the phosphate-binding protein dependent transport system which is composed of the phosphate-binding protein and probably three membrane-bound proteins (9). The binding protein dependent system is responsible for controlling the intracellular phosphate level. A mutation in any of the genes encoding components of the binding protein dependent transport system results in a defective transport system, a drop in intracellular phosphate, and the derepression of at least 20 different operons involved in phosphate transport and metabolism (10). Mutations in the genes encoding the membrane associated protein components of the transport system allow for the high level of production of the phosphate binding protein in strains bearing plasmids that express only the phosphate-binding protein (9).

Thus, to achieve a high level of production of phosphate binding protein in a strain that secretes this protein directly into the medium, the following are necessary:

- A mutant strain with a defective cell wall that leaks its periplasmic fraction into the medium

- The strain should have a defect in one or more of the membrane associated proteins of the binding protein dependent phosphate transport system.

- In addition, this strain should have a high copy number plasmid that carries the gene that encodes the phosphate binding protein, but not the gene that encodes the mutant membrane associated component(s) of the transport system.

Experiments to generate an overproducer strain that secretes high levels of phosphate-binding protein into the medium are in progress. An experiment that illustrates the high levels of phosphate-binding protein that can be secreted into the periplasm is shown in Figure 10. Growth on low phosphate of the strain that carries the gene for the binding protein on a plasmid results in a very high level of phosphate-binding protein in the periplasmic fraction. Approximately 70% of the entire periplasmic fraction is phosphate-binding protein.

Figure 10. SDS-PAGE analysis of the production of phosphate binding protein by cells grown in low versus high phosphate. Lane 1, periplasmic fraction (50 ug) of cells grown on high phosphate. Lane 2, periplasmic fraction (50 ug) of cells grown on low phosphate. PBP, Phosphate-binding protein.

The generality of the approach of solute removal by immobilized binding proteins has also been tested with immobilized glutamate-aspartate binding protein. Batch binding experiments gave results similar to those obtained with the phosphate-binding protein studies.

DISCUSSION

Proteins are generally thought of as rather fragile, unstable molecules. The existence of a special class of proteins that are resistant to proteases, boiling, conditions of very high and very low osmotic strengths and high and low pH values led us to consider the use of this class of proteins for the construction of cycling column adsorbers for the removal of specific solutes from waste steams. The first basic question was whether a regenerable adsorber could be designed that would provide efficient scrubbing of a solute from a stream. Our preliminary experiments with a small-scale adsorber containing immobilized *E. coli* phosphate-binding protein indicate that the basic technology is feasible.

The question of designing production strains that would generate large quantities of protein and secrete the overproduced protein directly into the culture medium also appear to be feasible. Our preliminary experiments indicate that the phosphate-binding protein can be secreted into the medium (Figure 9). Studies on the regulation of production of the phosphate-binding protein by others (e.g. 9) and verified in our experiments indicate that it should be possible to generate strains that secrete very large quantities of this protein directly into the medium.

The question of the diversity of proteins available for producing specific reactors is interesting. Table 1 lists the properties of the transport system associated binding proteins from *E. coli* and *S. typhimurium* that have so far been characterized. In addition, two cadmium binding proteins from *E. coli* have been described (11). In future experiments, we will investigate the feasibility of using one of the *E. coli* cadmium binding proteins in a cadmium adsorber. We have also described a general procedure for finding specific ligand binding proteins in virtually any organism (12). The procedure uses radiolabeled ligand to locate specific binding proteins in a two-dimensional gel of whole cell proteins. This procedure should be useful for isolating proteins with high affinity and specificity for other problem solutes.

SUMMARY

Proteins with high affinity and specificity for specific small molecules can be attached to solid phase supports and used to scrub the molecules of interest from solution. The adsorbed small molecules can be released from the protein simply by reversible denaturation of the protein. Recombinant DNA procedures can be used for the large-scale production of the specific proteins.

TABLE I[*]

BINDING PROTEINS FROM *Escherichia coli* AND *Salmonella typhimurium*

	Binding-Protein	Organism	Molecular Weight	K_D (uM)
Amino Acids	Ornithine	*E. coli*	28-33K	Arg .03-.1
	Lysine-Arginine Ornithine	*E. coli*	26-30K	Arg 0.15 Lys 3 Orn 5
		S. typhimurium	26K	
	Cystine	*E. coli*	27-28K	Cys 0.01
	Glutamine	*E. coli*	23-29K	0.15-0.3
		S. typhimurium	23K	
	Glutamate-Aspartate	*E. coli*	29-32K	0.8-6
		S. typhimurium	30K	Asp 1
	Histidine	*E. coli*	25-31K	0.8
		S. typhimurium	25K	0.15-1.5
	Leucine-Isoleucine-Valine-Threonine	*E. coli*	36.7K	0.2-2
		S. typhimurium	35-39K	Leu 0.43 Ile 0.15 Val 0.89
	Leucine-Specific	*E. coli*	37K	0.7
		S. typhimurium	34-38K	0.54
Peptides	Oligopeptide	*E. coli*	52K	
		S. typhimurium	52K	

TABLE I (continued)

	Binding-Protein	Organism	Molecular Weight	K_D (uM)
Sugars	Arabinose	*E. coli*	33K	0.2-2
	Galactose-(Glucose)	*E. coli*	32K	1
		S. typhimurium	33K	0.38
	Maltose	*E. coli*	40.7K	1
	Ribose	*E. coli*	29.5K	0.13
		S. typhimurium	29K	0.33
	Xylose	*E. coli*	37K	0.6
Anions	Citrate	*S. typhimurium*	28K	1-2.6
	Phosphate	*E. coli*	34K	0.8
	sn-Glycerol-3-Phosphate	*E. coli*	45K	0.2
	Sulfate	*S. typhimurium*	34.7K	0.02
Vitamins	B12	*E. coli*	22K	0.005
	Thiamine	*E. coli*		0.03-.1

Data derived from reference 13.

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DEGRADATION OF CHLORINATED BENZOATES UNDER A VARIETY OF ANAEROBIC ENRICHMENT CONDITIONS

by

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This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

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ABSTRACT

Degradation of chlorinated benzoates was studied under a number of anaerobic conditions. Enrichments were prepared by the addition of 3- or 4-chlorobenzoate to basal medium and 10% secondary anaerobic digested sludge. A 3-chlorobenzoate degrading consortium obtained from Dr. J. M. Tiedje was also studied as a model system for isolating and identifying organisms capable of dehalogenation.

Degradation of 4-chlorobenzoate was not observed after 29 weeks under any of the enrichment conditions. Degradation of 3-chlorobenzoate was observed in one of the sewage enrichments at 10 weeks. It was observed that the presence of nitrate inhibited growth and dehalogenation and that excess H_2 did not enhance the establishment of a 3-chlorobenzoate degrading consortium.

We were unable to isolate a novel 3-chlorobenzoate degrader from the consortium and have recently initiated genetic studies using the 3-chlorobenzoate degrading pure isolate DCB-1.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

INTRODUCTION

Halogenated organic compounds pose a serious environmental problem, because they enter the environment in substantial amounts, are toxic, and tend to accumulate in sediments and soils affecting both flora and fauna (1, 2, 3). Microbial degradation could be employed to remove these compounds *in situ* or during waste treatment. Aerobic degradation has been studied, but some of these compounds do not appear to be degradable aerobically (4, 5) and others are degraded in a manner in which highly toxic intermediates are formed (4, 6, 7a, 7b, 8, 9, 10, 11). Relatively little is known about anaerobic degradation of these compounds. However, recent investigations indicate that anaerobic degradation of a number of halogenated organic compounds occurs including halogenated benzoates (12, 13, 14) halogenated phenols (15, 16, 17, 18) and halogenated short chain aliphatic compounds (5, 19, 20). These studies have shown that some compounds which are not degraded aerobically are readily degraded anaerobically. In contrast to aerobic degradation, the first step of anaerobic degradation is the removal of the halogen (13, 14) leading immediately to the formation of a less toxic, more biodegradable compound. In the present study, we enriched for microbial anaerobic degradation of chlorinated benzoates under a number of anaerobic conditions in order to obtain a variety of organisms capable of dehalogenating and degrading chlorinated benzoates. We also obtained the 3-Cl-benzoate degrading consortium of Dr. J. M. Tiedje (21a) to use as a model system for isolating and identifying the organisms responsible for dehalogenation and degradation.

MATERIALS AND METHODS

ANAEROBIC METHODS AND MEDIA

The anaerobic techniques employed for the collection of inocula; preparation of media; and handling of enrichments and cultures were essentially those of Hungate (21b) as modified by Bryant (22) and Balch and Wolfe (23). The basal medium contained yeast extract (0.1%, w/v), B-vitamins, minerals, $NaHCO_3$, Na_2S reducing solution, resazurin redox indicator, and a 90% N_2-10% CO_2 gas phase (final pH 7.0). The terminal electron acceptor was CO_2 for methanogenic media, while 20mM Na_2SO_4, 15mM KNO_3 or 20 mM sodium fumarate was added for sulfate, nitrate or fumarate enrichments, respectively. Fermentative enrichments were prepared by adding the carbohydrates used in the Complete Carbohydrate medium (CC) of Leedle and Hespell (24).

MOST PROBABLE NUMBERS (MPN) ANALYSIS

Ten-fold serial dilutions were prepared with anaerobic dilution solution. One ml of each dilution was inoculated into triplicate tubes containing nine ml of the desired medium. The MPN was estimated from the number of tubes in each triplicate set which were positive for a desired characteristic (25).

SOURCE AND COLLECTION OF INOCULUM

The source of inoculum was secondary anaerobic digested sludge (Jackson Pike Plant, Columbus, Ohio). Inoculum was collected in a sterile 2 liter glass carboy containing a stir bar for mixing and sealed with a black rubber stopper fitted with a one-way gas valve to allow release of gas pressure.

ENRICHMENTS

Enrichments were prepared by adding sterile anaerobic 3- or 4-chlorobenzoate to the basal medium and 10% sewage sludge as inoculum. Enrichments were also prepared to determine the effect of hydrogen on the development of actively degrading consortia by adding 10% H_2 to enrichments after inoculation. Enrichments were incubated at 35°C. Degradation of the compound was followed over time by HPLC analysis. Once degradation was observed, the enrichments were transferred to fresh media and passed several times to stabilize the activity.

HPLC ANALYSIS

Benzoate, 3-Cl-benzoate and 4-Cl-benzoate were separated, identified and quantified by high pressure liquid chromatography (HPLC). A reverse phase C18 Lichrosorb column (10 u, 4.6 mm (ID) x 25 cm, Alltech Associates, Inc., Deerfield, Ill.) was used with a liquid phase of methanol:water:acetic acid (6:4:0.5) at a flow rate of 0.7 ml/min.

Fluid samples for HPLC analysis were collected aseptically and anaerobically via a 5 ml sterile syringe which had been flushed with anaerobic gas before sample collection. Samples were prepared for analysis by filtering through a 0.45 um Spartan-25 nylon filter (Schleicher & Schuell, Inc., Keene, N. J.) into new diposable glass test tubes. Slightly larger than 100 ul of sample was injected via Hamilton syringe into a 100 ul injection loop (Beckman/Altex; Berkeley, Calif.).

RESULTS AND DISCUSSION

DEGRADATION IN SEWAGE ENRICHMENTS

Degradation of 4-Cl-benzoate was not observed after 29 weeks of incubation under any of the enrichment conditions used (Table 1).

Persistence of 4-Cl-benzoate under methanogenic enrichment conditions had been previosuly reported (12) after 65 weeks of incubation.

3-Cl-benzoate was degraded in one of the duplicate nitrate enrichments without added H_2 at 10 weeks (Table 1). It had not shown degradation at 6 weeks. Degradation was observed at 10 weeks in the second duplicate as the 3-Cl-benzoate concentration had been reduced from 965 uM at 6 weeks to 689 uM at 10 weeks. All 3-Cl-benzoate had been degraded in the second duplicate by 23 weeks. The methanogenic and fumarate enrichments, which had not shown degradation at 10 weeks, showed complete degradation of 3-Cl-benzoate at 23 weeks. The sulfate and CCM enrichments did not show degradation after 23 weeks of incubation.

These results indicate that although anaerobic dehalogenation is a reductive process (13, 14), the presence of excess H_2 does not enhance the establishment of a consortium capable of degrading 3-Cl-benzoate. Microscopic examination of the enrichments revealed a mixture of gram-negative rods of varying lengths.

The nitrate enrichment showing degradation at 10 weeks was refed 800 uM 3-Cl-benzoate four times and showed an increased rate of degradation with each subsequent feeding (Figure 1a). A ten-percent transfer of this enrichment into fresh medium under methanogenic, sulfate-reducing and nitrate-reducing conditions indicated that nitrate was not required for degradation, and it was actually inhibitory (Figure 1b). Fifty percent transfers of the other enrichments into fresh medium without H_2 and with and without the terminal electron acceptor that had been present in the original enrichment indicated that neither H_2 nor the terminal electron acceptor were required for degradation. After 5.5 weeks of incubation the transferred fumarate enrichment showed complete degradation of 3-Cl-benzoate in the presence or absence of fumarate. At 10.5 weeks the methanogenic enrichments showed complete degradation, while the nitrate enrichments passed to medium containing nitrate showed no degradation. At 10.5 weeks only one of the duplicate nitrate enrichments passed to medium lacking nitrate showed degradation. These data support the conclusion that the presence of nitrate is actually inhibitory to degradation of 3-Cl-benzoate under these growth conditions.

DEVELOPMENT OF A 3-Cl-BENZOATE DECHLORINATING ENRICHMENT

A 3-Cl-benzoate degrading enrichment (14) was obtained from Dr. J. M. Tiedje. It was reported that the dechlorinating organism, which had been isolated in pure culture (21a), reduced nitrate to nitrite; grew somewhat poorly with pyruvate; and performed reductive dehalogenation of 3-Cl-benzoate. The consortium was inoculated (10%) into three media in an attempt to isolate the dechlorinating organism which was no longer available in pure culture. These included the basal medium plus 15 mM KNO_3; basal medium plus 15 mM KNO_3 and 50% H_2; and basal medium plus 0.3% pyruvate and 15 mM KNO_3. The 3-Cl-benzoate concentration was determined at 0, 3, 16 and 44 days (Figure 1c). At 44 days the culture containing

3-Cl-benzoate plus 15mM KNO_3 showed no detectable 3-Cl-benzoate, but a large peak was observed with a lower retention time (8.4 min versus 14 min) corresponding to 694 uM benzoate. The concentration of 3-Cl-benzoate in the enrichments containing either H_2 or pyruvate did not decrease during this time.

These data indicate that the 3-Cl-benzoate was being dechlorinated, but not degraded in the nitrate containing enrichment. After transfer into fresh medium, it was found that 3-Cl-benzoate was dechlorinated at a rate of 3.8 umoles/liter/hour and that as the 3-Cl-benzoate concentration decreased the benzoate concentration increased (Figure 1d).

A gram stain of the culture showed that the dominant cell type was a small gram negative coccobacillus predominately found in pairs and chains (Strain BG19, Figure 2a). Some large gram negative rods were also present. Cells with the distinct morphology of *Methanospirillum hungatei*, which were very apparent in the original consortium, were absent in the dechlorinating consortium. Methane was not detected. As a result of the degradation data, we can assume that under these conditions the benzoate degrader(s) and methanogens have been selected against and the dechlorinating organism(s) is one or both of the cell types present.

CHARACTERIZATION OF THE DECHLORINATING CONSORTIUM

A Most Probable Numbers (MPN) analysis of the dechlorinating consortium was performed (25) in order to determine total cells/ml versus dechlorinating organisms per ml. The consortium was diluted from 1×10^0 to 1×10^{-9} in anaerobic dilution solution and one ml was inoculated into nine ml of basal medium containing 3-Cl-benzoate (800 uM) plus 15 mM KNO_3. Optical densities were followed for two weeks and revealed a total of 2.5×10^8 cells/ml. HPLC analysis after 6 weeks indicated the presence of approximately 1×10^3 dechlorinating cells/ml. Thus, the dechlorinating organisms are present as a very minor portion of the total population indicating that direct isolation would be very difficult. Microscopic examination of the MPN cultures revealed that the small coccobacillus was the predominant cell type at all dilutions, but some large gram negative rods were present at the lower dilutions. A few small gram negative rods were also present at all dilutions. Since these rods could not be diluted away from the coccobacillus, they may be a morphological variation of the coccobacillus which had failed to divide properly. Their presence could also indicate an obligate syntrophic association between these two cell types under our growth conditions.

A series of media were inoculated with the dechlorinating consortium to determine the physiological capabilities of the organisms present. These included the basal medium lacking yeast extract with the following additions: 5 mM KNO_3 only; 5mM KNO_3 + 50% H_2; 5mM KNO_3 + 0.1% yeast extract; 5mM KNO_3 + 50% H_2 + 0.1% yeast extract; 5mM KNO_3 + 0.3% lactate; 5mM KNO_3 + 0.3% lactate + 0.1% yeast extract; 0.3% lactate + 0.1% yeast extract; 3-Cl-benzoate (800 uM) + 0.1% yeast extract; 3-Cl-benzoate +

0.1% yeast extract + 50% H_2; 50% H_2 + 0.1% yeast extract; 0.1% yeast extract only ; 3-Cl-benzoate + 0.1% yeast extract + 5mM KNO_3; 3-Cl-benzoate + 5 mM KNO_3; and 3-Cl-benzoate only. Hydrogen was added as a potential source of reducing equivalents for reductive dechlorination and lactate was added as an energy source for nitrate reducers present.

The maximum growth (600 nm) in triplicate tubes was followed for two weeks (Table 2). Growth did not occur in media lacking yeast extract. The presence of lactate was found to inhibit growth even in the presence of yeast extract. The best growth was observed in media containing H_2 in addition to yeast extract and the presence of nitrate tended to inhibit growth. These data indicate that organisms with a hydrogenase were present in the consortium and that despite being a nitrate enrichment, nitrate was not only not required for growth, but was inhibitory.

After 6 weeks HPLC analysis indicated that dechlorination occurred in all media containing 3-Cl-benzoate, except 3-Cl-benzoate + KNO_3 and 3-Cl-benzoate only. A concomitant increase in the benzoate concentration was observed in those media showing dechlorination. Thus, despite a lack of significant growth, dechlorination occurred in the medium containing H_2 + 3-Cl-benzoate, but lacking yeast extract. These data suggest that reducing equivalents for reductive dehalogenation are obtained from the yeast extract component, and only when reducing equivalents in the form of H_2 are provided will reductive dehalogenation occur without yeast extract or some other suitable compound. These data also indicate that nitrate is inhibitory to dechlorination, as well as growth.

Microscopic examination of those cultures containing hydrogen indicated gram negative rods of varying shape and lengths were predominant. Other cultures contained the small coccobacillus as the predominant cell type. Few cells were observed in the cultures showing little or no growth. In the defined H_2 + 3-Cl-benzoate culture which dechlorinated 3-Cl-benzoate, but showed little growth, a mixture of the gram negative coccobacillus and large rod were seen. In this case the large rod comprised approximately 50% of the population, which is a much higher percentage than the original dechlorinating consortium.

ISOLATED STRAINS PRESENT IN THE DECHLORINATING CONSORTIUM

Since dehalogenation did not occur in the MPN cultures containing the coccobacillus, but did occur in the lower dilutions containing the large gram negative rod, it is likely that the rod, and not the coccobacillus, is the dechlorinating organism. Since a large rod was present in greater numbers in the defined H_2 plus 3-Cl-benzoate medium, in which dehalogenation occurred, we attempted a direct isolation of the dechlorinating organism from this culture. The culture was streaked onto 3-Cl-benzoate plates plus yeast extract and was incubated with and without H_2. After two weeks, 102 colonies were picked into 3-Cl-benzoate plus yeast extract broth. Cultures were incubated for one week and cellular morphology and dehalogenation were determined. The majority of cultures contained the coccobacillus previously described, but five other morphological types were isolated. These included two coccobacillus which differed slightly from the dominant cell type. Strain BG95 (Figure 2b) was more spherical and strain BG29 (Figure 2c) did not form pairs, while chains were only periodically observed. Three gram negative rods were isolated, including: strain BG2, a medium rod with pointed ends (Figure 2d); strain BG49, a medium rod with round ends (Figure 2e); and a long thin rod similar to *Methanospirillum hungatei*. The presence of H_2 had little effect on the type of cell isolated. The 3-Cl-benzoate concentration in cultures of each morphological type was determined weekly for one month, but dechlorination was not observed. A second attempt at isolation of a dechlorinating organism resulted in the isolation of two more types of gram negative rods, including a medium rod (strain BG131) which was usually found in pairs (Figure 2f) and a long oval rod (strain BG170) which tended to divide unevenly (Figure 2g). After one week 3-Cl-benzoate had not been dechlorinated by either of these isolates. It is possible that dechlorination requires the presence of one or more of these isolates due to some type of cross-feeding and may account for the lack of dechlorination in pure culture. This could be determined by growing these organisms in various combinations in 3-Cl-benzoate medium.

Since this time, we have received the dechlorinating culture DCB-1 in pure culture from Dr. J. M. Tiedje (21a). The DCB-1 culture has been shown to also dehalogenate 3-iodobenzoate and is thought to be related to the genus *Desulfovibrio*. The pure culture has been propagated and shown to dehalogenate 3-chlorobenzoate. Further studies will be directed at elucidating the genetic basis for the dechlorinating activity. A rapid screening procedure for the detection of dehalogenation and methods for extracting plasmid and chromosomal DNA will be developed.

Extraction of DNA from the DCB-1 strain will be based on methods used by other investigators working with *Desulfovibrio* strains. These organisms have proven resistant to milder cell disruption methods including lysosome, SDS and protease treatments. The method currently producing the best results utilizes treatment with RNAse A followed by disruption in a French Press and phenol:chloroform extraction. The extracted chromosomal DNA will be sheared, size fractionated and then cloned into an *E. coli* host organism in order to establish a clone bank of the entire chromosome. Any plasmids isolated will be analyzed by treatment with restriction endonucleases and cloned into the same *E. coli* host. The vector pDP1 to be used in the cloning experiments is a hybrid constructed from pDG5 and the *Bacteroides* clindamycin resistance plasmid pCP1. This vector replicates in both *E. coli* and *Bacteroides fragiles* and contains the RK2 *ori* T sequence. With the helper plasmid, pRK231, fragments cloned and banked into *E. coli* can be reintroduced into the original host or other appropriate anaerobe hosts, such as *Bacteroides* to check for expression of the dehalogenation activity.

A rapid screening method for the detection of dehalogenation is essential for the detection of recombinant clones expressing the dehalogenating trait. A method is currently being developed that is based on the release of iodide that accompanies dehalogenation of 3-iodobenzoate.

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TABLE 1. DEVELOPMENT OF 3-Cl-BENZOATE DEGRADING CONSORTIUM UNDER VARIOUS ENRICHMENT CONDITIONS

Enrichment Type	Time (Weeks)[1]	
	no H_2	$+H_2$
3-Cl-benzoate		
methanogenic	10-23[2]	10-23
sulfate	>23[3]	>23
nitrate	6-10	10-23
fumarate	10-23	10-23
CC	>23	>23
4-Cl-benzoate		
methanogenic	>29	>29
sulfate	>29	>29
nitrate	>29	>29
fumarate	>29	>29

1) Weeks of Incubation Before Degradation Observed

2) Degradation not Observed at 10 Weeks, but Apparent at 23 Weeks

3) Degradation not Observed at Last Sampling

TABLE 2. GROWTH AND DECHLORINATION OF 3-Cl-BENZOATE BY DECHLORINATING CONSORTIUM UNDER VARIOUS CONDITIONS

Additions[1]	Growth[2]	3-Cl-benzoate (μM)	Benzoate (μM)
KNO_3	0.05	—	—
H_2/KNO_3	0.05	—	—
YE/KNO_3	0.22	—	—
$H_2/KNO_3/YE$	0.32	—	—
Lactate/KNO_3	0.08	—	—
Lactate/KNO_3/YE	0.05	—	—
Lactate/YE	0.07	—	—
3CB/YE	0.24	147.3	630.5
H_2/3CB/YE	0.47	0	752.0
H_2/YE	0.42	—	—
YE	0.22	—	—
3CB/YE/KNO_3	0.32	384.4	422.6
3CB/KNO_3	0.05	815.9	65.8
H_2/3CB	0.06	0	748.0
3CB	0.05	757.5	58.2

1) Basal Medium Without Yeast Extract Plus the Additions Indicated (See Text)
2) Maximum Optical Density (600 nm) After Two Weeks

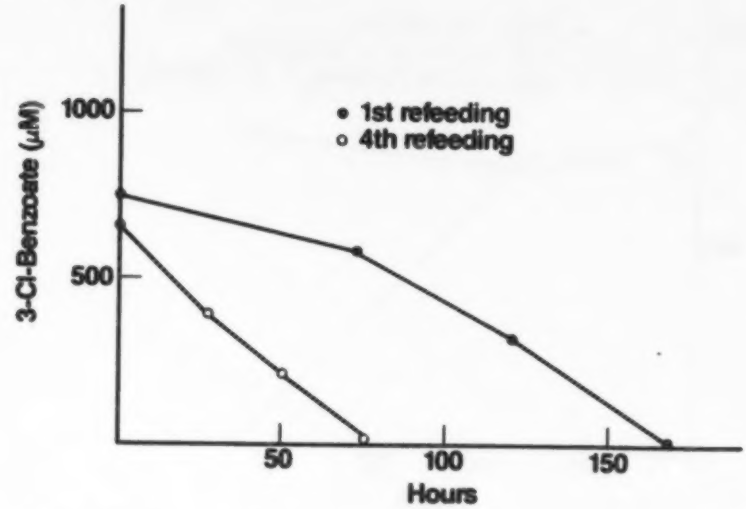

FIGURE 1:a. DEGRADATION OF 3-Cl-BENZOATE IN COLUMBUS SEWAGE BY 3CB/NO_3 ENRICHMENT.

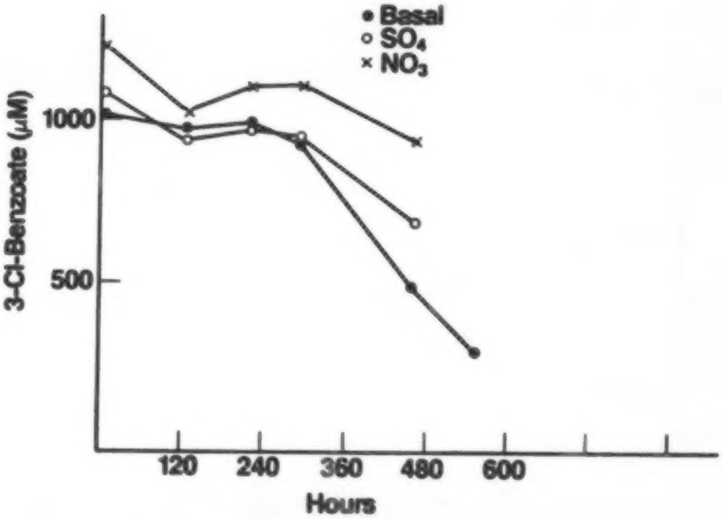

FIGURE 1:b. DEGRADATION OF 3-Cl-BENZOATE IN BASAL, SULFATE AND NITRATE MEDIUM INOCUBATED WITH 10 PERCENT COLUMBUS SEWAGE ENRICHMENT THAT DEGRADES 3-Cl-BENZOATE.

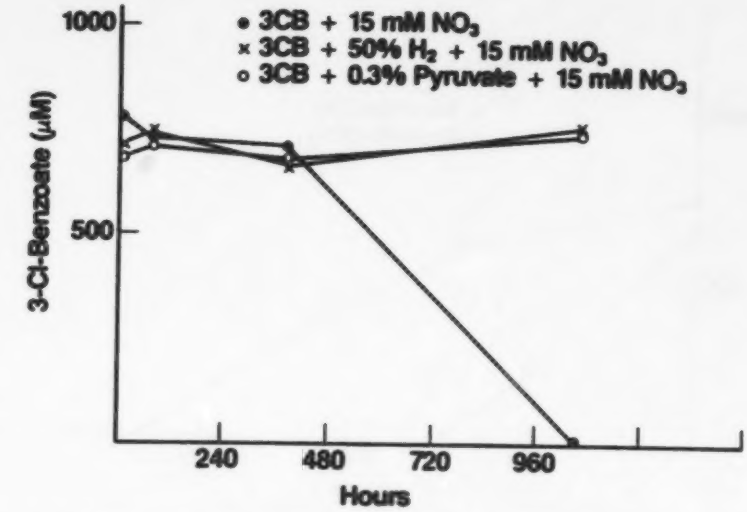

FIGURE 1:c. DEVELOPMENT OF DECHLORINATING 3-Cl-BENZOATE/NO_3 ENRICHMENT FROM T-ENRICHMENT INOCULUM.

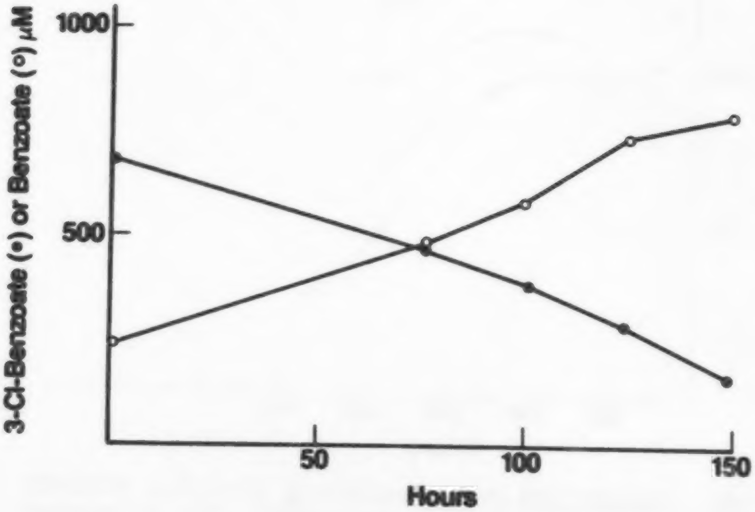

FIGURE 1:d. DECHLORINATION OF 3-Cl-BENZOATE BY T-(NO_3) ENRICHMENT.

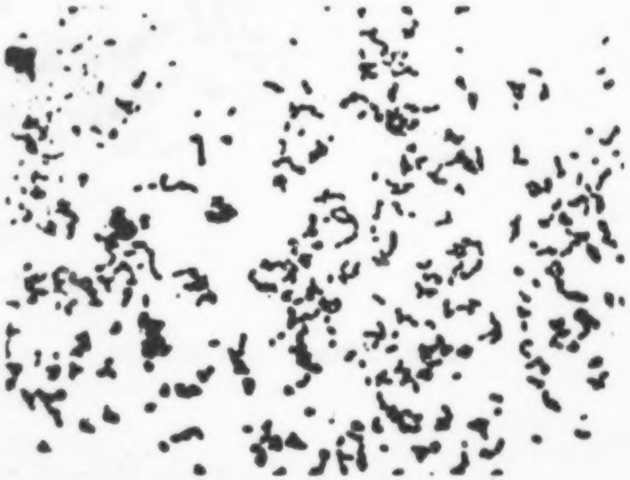

FIGURE 2:a. STRAIN BG19.

FIGURE 2:b. STRAIN BG95.

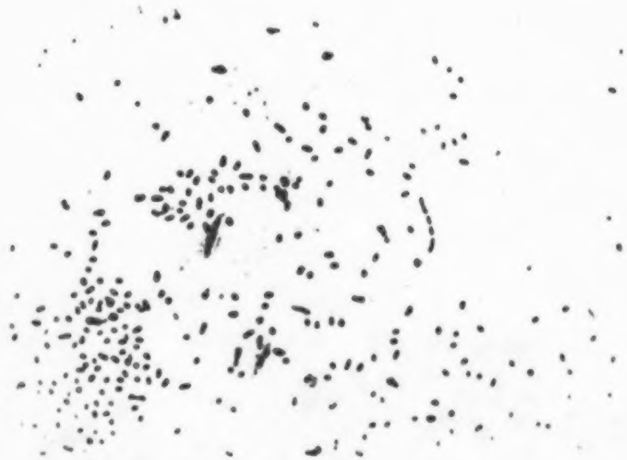

FIGURE 2:c. STRAIN BG29.

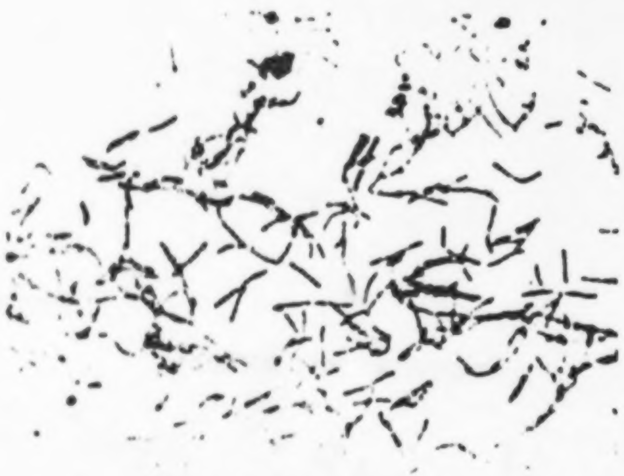

FIGURE 2:d. STRAIN BG2.

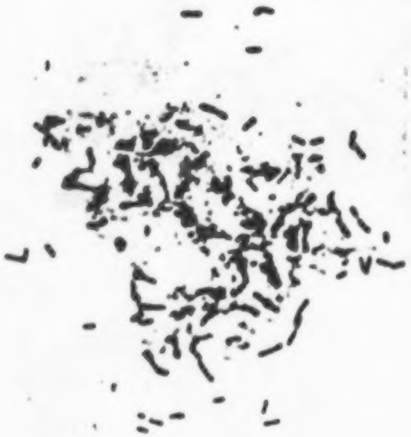

FIGURE 2:e. STRAIN BG49.

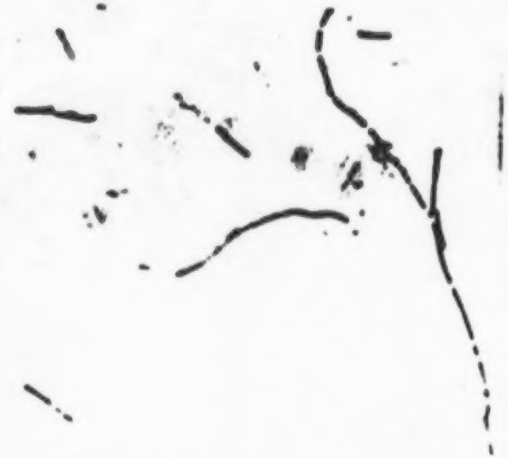

FIGURE 2:f. STRAIN BG131.

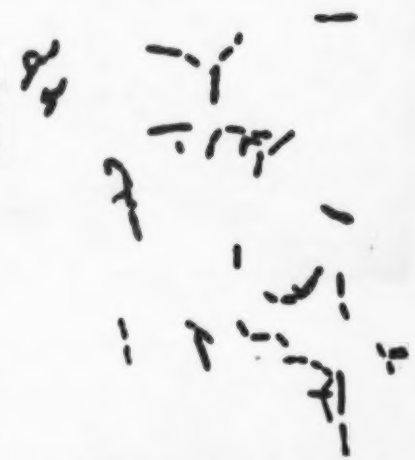

FIGURE 2:g. STRAIN BG170.

ASSESSMENT OF BIOAUGMENTATION TECHNOLOGY AND EVALUATION STUDIES ON BIOAUGMENTATION PRODUCTS

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ABSTRACT

An overview of bioaugmentation technology and its application to biological wastewater treatment through the use of biocatalytic products is presented. The report defines and characterizes bioaugmentation, discusses the principles of biological augmentation, describes the methodology for the production of biocatalytic microbial and enzymatic additives through selective adaptation and mutation and classifies the varied groups of bioaugmentation products.

Manufacturers' assessment of bioaugmentation possibilities and the range of treatment benefits through the use of their biocatalytic products is counterweighed by a discussion of the shortcomings of bioaugmentation technology as revealed by laboratory and field scale research studies.

Finally, the report discusses the ongoing evaluation studies funded by the U.S. Environmental Protection Agency (EPA) using developed label verification and performance (efficacy) testing protocols. The project goals are: to place the use and characterization of these types of products on a scientific basis; to arrive at consensus standards for label verification/package contents and performance of these products and to improve the credibility of bioaugmentation technology through the use of standard testing protocols for evaluation of the biocatalytic products.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

INTRODUCTION

Over the past twenty years there has been an increased interest in the use of nonstructural approach for improvement of effluent quality in municipal and industrial wastewater treatment without a major upgrade in the treatment process. Bioaugmentation approaches, such as addition of enzymes to improve flocculation in a physical/chemical treatment process or bacterial augmentation of an activated sludge process to decrease the level of pollutants in the treated discharge may provide such a nonstructural approach. During the same period of time, increased reference has appeared in the literature on the ability of specialized bacterial cultures to produce improvements in biological waste treatment. These improvements include: decreased sludge production; reduced foam in the aeration process; color reduction; suppressed filamentous organisms in activated sludge; improved response from a peak load; and an overall improvement in effluent quality.

Various types of enzymes, dried bacterial cultures, by-products of bacterial fermentation products, biocatalytic additives, and combination of microbiological cells and their fermentation products have been developed. Suggested applications of these products include solubilization of grease; breaking up the scum and dissolving the fat in anaerobic digesters; improving the performance of septic tanks and leaching fields; increasing the biochemical oxygen demand (BOD) removal efficiency of primary sedimentation, activated sludge and trickling filters; controlling foaming in aeration tanks; unclogging sewers, pipes and trickling filter media; increasing performance of stabilization ponds; and deodorizing sewers, septic tanks and small treatment systems. In addition, the added bacterial cultures are said to improve performance of overloaded plants by increasing kinetic rates.

The beneficial effects on the performance of biological waste treatment processes associated with the use of such a technique has not always been clearly demonstrated. Reports on application of bacterial additives have often overstated the advantages or presented selected examples of success. In addition, failure to use a full scale control when evaluating these products makes documentation of their advantages difficult. Under the evaluation procedures, careful plant operation during the testing periods could cause the observed improvements in efficiency.

It is the purpose of this report to review the bioaugmentation industry's applications and uses of bioaugmentation products and their technological approach in wastewater treatment; to present the manufacturers assessment of bioaugmentation possibilities and the range of treatment benefits achieved by the technology; to present pertinent research results and critiques by critics of the bioaugmentation approach; and finally, to describe the ongoing evaluation studies on bioaugmentation products funded by the U.S. EPA at the National Sanitation Foundation.

BIOAUGMENTATION TECHNOLOGY AND ITS APPLICATION

As practiced today, bioaugmentation involves the addition of selected microorganisms to a naturally occuring population in a wastewater treatment system to increase the biological activity of the system. The bioaugmentation technologists state that bioaugmentation is very cost competitive with other upgrading approaches in wastewater treatment, and by actually degrading pollutants (usually to carbon dioxide and water), solves the pollution problem instead of transferring pollutants to another location. A variety of bacterial preparations (including some with added nutrients or enzymes) are presently available for bioaugmentation in treatment of municipal, industrial, and agricultural wastes and wastewaters. The addition of specific cultures of microorganisms to a process, known as bioaugmentation, is a commonly used procedure in many industries including brewing, cheesemaking, wineries, dairy products, pharmaceutical and fermentation, to mention a few. The majority of fields using biological processes rely upon this technique. Bioaugmentation products suppliers indicate that wastewater treatment constitutes the largest user of biological processes.

THE BIOAUGMENTATION CONCEPT

The concept of bioaugmentation has been variously defined. Flow Laboratories defines bioaugmentation as "the addition of specific organisms into an environment for specific purposes....to assure consistency of quality by eliminating the opportunity of unfavorable, happen-stance organisms to cause undesirable effects." This definition is thus applicable to the use of bacterial culture in the beer, wine, dairy, and pharmaceutical industries as well as to their use in wastewater treatment. Dominance of the introduced bacteria is the implied objective. In contrast, Reliance Books defines biological augmentation as process which "augments the existing bacterial population with bacteria that are capable of higher rates of organic oxidation or that are capable of degrading organic compounds that have previously been nondegradable." This definition is followed by the qualifying statement that "the object is not to replace the existing bacteria but to supplement them for improved efficiency." The differences between these two definitions is probably more of a semantic than of a scientific nature as the methods and objectives of these two companies are essentially identical.

The principle of ubiquity (Figure 1) states "that organisms necessary for satisfactory wastewater treatment are available in any soil or the wastewater stream itself, and that the organisms capable of using a specific organic substrate in the waste, are selected out within a total population through natural selection processes." In contrast to the ubiquity principle stressing the natural selection process, proponents of the bioaugmentation approach stress that organisms are normally not selective toward specific organic substrates but rather handle an array of various substrates within their

THE UBIQUITY PRINCIPLE

"...all types of bacteria are available at all times everywhere..."

hence:

Natural population selection mechanism will always result in the right biological culture for treatment of a given waste.

Figure 1. The Ubiquity Principle.

metabolic capabilities. The basis of biological supplementation (bioaugmentation) is the need for added bacteria, capable of handling specific problem organic substrates, to achieve an artificial dominance in the microbial population that will improve treatment performance.

With programmed bacterial supplementations, sufficient quantities of selected bacteria are added to the system initially, and on a repetitive basis, to produce a competitive number of the supplemented organisms. In this way, the augmentation changes the natural population distribution and the maintenance dose maintains the necessary numerical advantage of the desirable organisms. Maintenance doses are used on a variable basis, depending upon the nature of the system. The proponents claim that bioaugmentation thus offers an effective means of controlling the nature of the biomass.

In the view of bioaugmentation technologists, the various types of bacteria incorporated in the formulations differ from those which find a natural dominance through the natural selection processes within a typical treatment system. One is not simply adding numbers of bacteria but changing the quality and characteristics of the existing biomass. The effective formulations are based on selected species and strains which have an enhanced ability to breakdown problem organic substances compared to bacteria that achieve natural dominance within a given system. Sustenance treatments, however, are not normally required in instances where competitive organisms are not routinely entering the system or in chemical waste disposal processes in which hard to handle chemical wastes are continuously treated.

Bioaugmentation researchers present cases which strongly support the conclusion that, although microbial ubiquity and natural population selection and adaptation are effective in problem situations of a continuous nature, most biological wastewater treatment systems regularly face intermittent problem situations to which natural adaptive processes are unable to respond in a timely basis. According to them, blends of specialized preselected, adapted, mutant microbiota can reduce the response time of such biological systems and thus improve their performance and stability. They maintain that a combination of effective microbial applications technology, appropriate system design and informed systems operations and maintenance (biomass engineering) have enormous potential for yielding cost-effective waste treatment for industry.

METHODOLOGY FOR THE PRODUCTION OF BIOCATALYTIC MICROBIAL ADDITIVES

In general, bioaugmentation organisms are mutated by chemical in situ mutation, and not by recombinant DNA techniques. With the latter technique, the organisms are lysed, the genetic feature of interest is isolated, and then it is reintroduced into living organisms. With chemical in situ mutation, most of the organisms are likely to be rare mutants. That is, a capability that was already present in living organisms (often Pseudomonas aeruginosa) is enhanced by mutation as much as a thousandfold. Unlike the genetic engineering method which involves gene splicing and plasmid transfer technology, conventional mutational methods involving strain selection and chemical mutagenesis are being used in the production of randomly achieved microorganisms specialized for a particular task. In a treatment situation, the organisms simply increase the degradation rates in the system.

Although the microbes that the bioaugmentation industries market for specialized waste treatment are, in a sense, genetically engineered, they are produced by "natural" methods. In essence, the process by which evolution devises organisms specially adapted to a given environment is accelerated in the production of the bioaugmentation additives.

First, product developers collect organisms, often from special sites where natural selection has already favored microbes adapted to unusual conditions. The organisms are then grown in the laboratory and are subjected to enrichment culture techniques in a medium containing the pollutant they are supposed to degrade or under condition that enhanced the desired treatment effect. This process selects the organisms that accomodate best to the medium's chemistry and available nutrients, i.e., or to appropriate environment conditions.

Scientists speed up the organisms' adaptation to the specialized environment or substrate by increasing their mutation rates using tools such as radiation and chemical mutagens. The object of the mutagenesis, is not necessarily to grossly alter the organisms' natural metabolism but to accelerate the rates of enzymatic activity. The genetic changes induced by the mutagens result in some microbes that are better at degrading a particular pollutant or producing a desired treatment effect than their predecessors.

After this trial-and-error process, the best adapted mutants are grown in large quantities. The companies may market their microbes as dried powders or liquids. The dried products usually contain mixtures of organisms adapted to a particular waste treatment task, along with additives such as wetting agents and emulsifiers to aid in dispersion and nutrients. To activate the microbes, warm water is added to the contents and the mixture is stirred. Liquid products are suspension of bacteria, their metabolic products, enzymes and nutrients.

Selective Adaptation and Mutation

Researchers producing the bioaugmentation additives, maintain that during the process of selective adaptation and mutation, these microbes are "evolving" under conditions other than those found in the "real world". Perhaps most significantly, they are not subjected during this process to the strongest competitive selective pressures of the "real world". As a result, although enhanced abilities to degrade given substrates can be obtained, this is normally accomplished at the cost of those qualities which provided the original microbe with "real world" survivability under such pressures. When the new strains are reintroduced to the competitive environment of the natural ecosystem (particularly if their favored substrates are either absent, only intermittently present or present in low concentration), their survivability may be marginal. Thus their longevity under such conditions may be significantly reduced, although not terminated. This means that generally regular reinoculation is required to maintain them in a given "natural" environment. It also means that they tend to be "selected out" of the natural ecosystem when conditions favoring their survival are eliminated (e.g. the removal of a preferred substrate).

CHARACTERIZATION OF BIOAUGMENTATION PRODUCTS

Currently there are approximately between 60 to 70 industrial concerns and suppliers in the United States, manufacturing and representing the competitive bioaugmentation products, consisting of either microorganism formulations, enzyme preparations, or combinations of bacterial cultures and enzymes. Some of the larger industries include Polybac, a subsidiary of Cytox Corporation; Sybron Biochemical, a division of Sybron Corporation; Environmental Cultures Division of Flow Laboratories Inc.; Miles Laboratories, Inc.; Bioscience Management, Inc; General Environmental Sciences Corporation; Industrial Microgenics, Ltd; Reliance Brooks; Jet, Inc.; Solmar Corporation; Materials Bio-Science Corporation; Worne Biochemicals, and J.T. Baker (Environmental Protection).

Polybac supplies engineered microbes for commercial waste treatment to food processors, chemical manufacturers, petroleum refiners, petrochemical plants and for hazardous waste cleanup. Sybron sells bacterial cultures to industries ranging from food processing to steel coking, and boasts that one of its pseudomonads was the first bacterial life form to be patented. The company maintains that its organisms include some that will degrade Arochlor 1260, one of the most highly chlorinated of the polychlorinated biphenyls (PCBs), generally considered non-biodegradable. Enviroflow specializes in municipal water and sewage treatment.

General Environmental Sciences Corp. and Jet, Inc. market liquid suspensions of bacterial cultures. One of these formulations, Liquid Live Microorganisms (LLMO), is produced by growing bacterial cultures to maturity, and chemically inducing a dormant state, presumably by addition of a growth or metabolic inhibitor. These cultures supposedly can be held in this suspended state for an indefinite period of time, and full population recovery is expected when the culture is diluted and suitable nutrients become available. Sybron markets both wet and dry cultures whereas the other companies offer dry cultures only. Dry cultures require strictly dry conditions for storage and cannot be frozen. Maximum shelf life is 1 - 2 years. Industrial Microgenics offers immobilized cell preparations in which the microorganisms are supported by semi-solid or granular substrates.

Many industries, such as Reliance Brooks, Sybron, Industrial Microgenics, and Polybac offer "mutant" bacterial cultures, but details regarding the parent strains and the genetic engineering techniques used to induce and select mutations are apparently of a proprietary nature and are not made available. However, Industrial Microgenics does present a brief description of a treatment process involving 5-bromouracil and UV-radiation, which is used to induce mutations in bacteria isolated and selected for tolerance of high levels (approx. 1000 ppm) of various compounds (e.g. phenols). Unlike the above industries, Enviroflow uses organisms selected from the environment without modifying them via mutation.

In all cases the product literature warns against exposure of bacterial cultures to toxic levels of heavy metals, especially chromium. Polybac specifically recommends that the hexavalent chromium concentration not exceed 2 ppm in the treatment system. Bacterial cultures or similar products for metal waste treatment or metal recovery evidently have not been developed or marketed to date. Most presently available formulations are designed for biological degradation of organic wastes with a few formulations for treatment of cyanide or cyanate waste, and control of H_2S emissions.

Bacterial cultures for bioaugmentation of wastewater treatment are being used when one or more of the following problems (Figure 2) occur: foul odors persist; temperature, or hydraulic or chemical loading changes drastically; grease and oil accumulate, resulting in blockage or formation of surface scum and reduction of oxygen transfer rates; the waste is a chemical inhibitor of biological growth or metabolism; the nutrients needed for bacterial growth are deficient; BOD, COD, suspended solids, color, or nitrogen levels exceed effluent standards; algae or duckweed growth occurs; build-up of solids or excessive foaming occurs; corrosion of metals occurs due to the action of Thiobacillus organisms in the presence of H_2S; floating sludge, and high effluent solids.

Only a few companies provide the genera of bacteria present in their culture. These genera of bacteria used for bioaugmentation are listed in Table 1. In no instance, are species names or other taxanomic information provided. In addition to the bacteria listed, several fungi are cited including Rhizopus, Aspergillus, Candida, Myrothecium and Tricho-Cellulomonas.

COMMON SIGNS OF BIOLOGICAL SYSTEM STRESS

- -FOAMING
- -FLOATING SLUDGE
- -BOD and COD BREAKTHROUGH
- -HIGHLY TURBID EFFLUENT
- -LACK OF HIGHER LIFE FORMS
- -INCREASED OR DECREASED RESPIRATION RATES
- -HIGH EFFLUENT SOLIDS

Figure 2. Common signs of biological system stress.

Bacillus is probably the most important bacterium present in these cultures because of the diversity of substrates it can utilize, and because of its ability to form heat-resistant spores.

General Environmental Science Corp. provides a number of documents addressing the potential human health hazards of bioaugmentation. According to researchers at Case Western Reserve University, experiments failed to show any toxic effects on mice injected with LLMO. Inspection by the USDA is required for certification of the absence of Salmonellae and other pathogenic microorganisms. The USEPA presently does not require registration of LLMO and for that matter any other bioaugmentation product on the market for wastewater treatment.

THE CONTROVERSY ON BIOAUGMENTATION APPLICATIONS

I. Manufacturer's Overview of the Treatment Benefits Achieved by Bioaugmentation Technology

According to L.T. Davis (1) a range of treatment benefits have been achieved with bioaugmentation technology: generating good bioactivity, especially in very toxic systems; greatly increasing the rate of degradation, and sometime precluding the need to build a bigger treatment system; biodegrading compounds that had previously passed through the system; maintaining efficient bioactivity at low temperatures to yield significant savings on heat costs; sustaining efficient bioactivity with lower aeration costs by means of more efficient oxygen utilization; withstanding variable waste streams and shock loadings, thereby promoting greater system stability, recovering quickly from a "kill" situation; reducing foam; and enhancing sludge settleability.

TABLE 1. GENERA OF BACTERIA USED FOR BIOAUGMENTATION

Genus	Gram Stain	Oxygen Requirement	Spore Formation	Shape
Actinomycetes	+	microaerotolerant	no	mycelial
Aerobacter	-	facultative anaerobe	no	rod
Arthrobacter	+	strict aerobe	no	irregular
Bacillus	+	strict aerobe or facultative anaerobe	yes	rod
Cellulomonas	+	strict aerobe	no	irregular
Desulfovibrio	-	strict anaerobe	no	curved rod
Mycobacterium	+	strict aerobe	no	mycelial
Nitrobacter	-	strict aerobe (ammonia oxidizer)	no	pear-shaped
Nocardia	+	strict aerobe	no	mycelial
Pseudomonas	-	strict aerobe	no	straight or curved rod
Rhodopseudomonas	*	photosythetic	no	rod

*** Gram stain is used to distinguish non-photosynthetic bacteria only.**

Davis maintains that bioaugmentation treatment has yielded impressive results in a number of wastewater applications in chemical and pharmaceutical industry. Biocatalysts are added to: withstand organic overloading; maintain efficiency at lower temperatures in winter; and successfully compete against filamentous growth. Heavy initial dosage is needed to establish a dominant place in natural population, a maintenance dosage is also required to compete with naturally occuring organisms and maintain a population in the system, and to compensate for back mutation, whereby some of the organisms' potency is gradually lost. Bioaugmentation researchers have isolated from soils contaminated with Arochlor 1260, and mutated a group of organisms that exhibit a high degradation rate of this PCB. Treatment in activated systems augmented with these microbiota reduced 100 to 400 ppm PCBs to undetected levels. Mutated cultures have also been produced that successfully degrade the analogs of dioxin (2,4,7,8 tetrachlorodibenzoparadioxin).

Grubbs (2) describes the use of bioaugmentation products in: waste treatment plants, enhancing BOD_5 removal and COD removal rates; in aerobic and anaerobic digesters, improving digester performance; pretreatment of industrial wastes; and hazardous waste applications. According to Grubbs (3) successful environmental applications of bioaugmentation technology were made in domestic sewage situations, through enhancement of BOD and COD removals, control of grease, improved digestion of sludge, reduction of odors and control of H_2S emissions; industrial wastewater pretreatment, by improving BOD and COD removals; and treatment of petroleum based wastes and hazardous wastes. Report has also been made by Grubbs (4) on the use of bioaugmentation products in enhancing BOD_5 removal rates in final effluent for activated sludge wastewater treatment for potato wastes, corn product wastes, and in increasing % BOD removal for dairy wastes across aerated lagoon systems.

Bacterial supplementation has been shown (4) to: improve BOD_5 removals; increase sludge settleability; lower sludge volumes; eliminate grease mats, control malodors; reduce H_2S corrosion; improve digestion of solids; improve digester operations; provide much quicker recovery from upsets due to shock loadings or mechanical failures; prevent malodors from lagoon inversions; clean grease in collection systems; restore percolation of fields, percolation ponds, etc., which are plugged from organic matter, and give more predictable results. Factors limiting bioaugmentation can be enumerated as follows: poor engineering design and operational practices; very low dissolved oxygen levels, which will stimulate filamentous growth (*Sphaerotilus* species); caustics, chlorine, bactericides; and variations in hydraulic organic loadings.

Numerous articles (2, 4-16) have described the successful use of cultures to control grease within sewage collection systems to reduce sewer-line clogging and to improve sewer-line systems treatment. Reductions of odors and control of hydrogen sulfide emissions (2,4,5,9,10, 11, 17-20) have also been widely reported. Improvements in BOD_5 and COD removals (2,4-7,9,10,21-26) through the use of bacterial supplements in industrial wastewater treatment applications have been reported in the literature.

Worne (25) reported on the activity of adapted and mutant microorganisms supplemented in the biological treatment of industrial wastes and on their use in the degradation of specific organic compounds. The use of adapted and mutated microbiota offers advantage of immediate activity with pre-computed levels of biochemical activity for removal of various toxicants in contrast to the time required for adaptation in nature of wild strains. The concentration of these biocatalytic additives assures the formation of significantly higher concentration of enzymes, and establishes higher rates of efficiency and more rapid velocities of degradation. Biodegradation of aryl halides, halophenols, aliphatic amines and aryl amines by parent and adapted/mutant microbiota is shown in Tables 2, 3, 4 and 5, respectively. Biodegradation of ABS-LAS surfactants by parent and adapted/mutant microbiota is expressed graphically in Figures 3, 4 and 5. Table 6 provides biodegradability data on various inorganic and organic cyanides.

The use of adapted mutant microbiota for the enhancement of refinery effluent treatment (26-33) is well documented in the literature. The impact of a mutant bacterial aid on the performance of an activated sludge process was illustrated in a full scale application conducted on a refinery wastewater by McDowell and Zitrides (32). Two identical activated sludge systems operating in parallel provided control and trial units during a three month investigation. The process receiving the mutant bacteria provided a 32 percent improvement in effluent quality as measured by total organic carbon (TOC). In addition, probability distributions of the treated effluent quality indicated less variability in the trial unit. The authors suggested that the mutant organisms accelerated the response of the activated sludge which resulted in a less variable effluent quality. The mutant organisms increased activated sludge performance, resistance to upsets and shock resistance, as illustrated in Figures 6,7 and 8, respectively. A mathematical model using Monod kinetics was proposed to describe the transient response time of the activated sludge. By varying the model kinetic constants for maximum specific growth rate, μ-max, and the half velocity coefficient, Ks, the sensitivity of the model to bacterial modification was illustrated in which a lowering of Ks and an increase in μ-max provided a faster response of the activated sludge to a shock load. Although the authors proposed that the mutant microorganisms produced such a result, the parallel activated sludge processes in this study were not used to test the authors' hypothesis.

Tracy and Zitrides (31) discuss the enhancement of process kinetics in refinery effluent treatment units containing Phenobac cultures. Whereas the control units show a decreasing coefficient rate with increasing TOC, suggesting inhibition of the biomass, the coefficients in the Phenobac supplemented units, show no inhibitory effects and remain constant with increasing TOC loadings, suggesting greater resistance of Phenobac supplemented cultures to substrate shocks. The comparison of kinetic constants between treated and control units is illustrated in Table 7.

Enhancement of the treatment of petrochemical wastes by adapted mutant microbiota has been reported in several articles (34-37). Use of freeze-dried mutant microorganisms to improve the effluent from a pure oxygen activated sludge process treating petrochemical wastewater has been studied

TABLE 2. DEGRADATION OF ARYL HALIDES BY A MUTANT PSEUDOMONAS SP. (a) 30°C

Compound	Conc	Ring Disruption %		Time in Hours	
		Parent	Mutant	Parent	Mutant
Monochlorobenzene	200 mg/l	100	100	58	14
o-Dichlorobenzene	200 mg/l	100	100	72	26
m-Dichlorobenzene	200 mg/l	100	100	96	28
p-Dichlorobenzene	200 mg/l	100	100	92	25
1,2,3-Trichlorobenzene	200 mg/l	87	100	120	43
1,2,4-Trichlorobenzene	200 mg/l	92	100	120	46
1,3,5-Trichlorobenzene	200 mg/l	78	100	120	50
1,2,3,4-Trichlorobenzene	200 mg/l	33	74	120	120
1,2,4,5-Tetrachlorobenzene	200 mg/l	30	80	120	120
Hexachlorobenzene	200 mg/l	0	0	120	120

TABLE 3. DEGRADATION OF HALOPHENOLS BY A MUTANT PSEUDOMONAS (a) 30°C

Compound	Conc	Ring Disruption %		Time in Hours	
		Parent	Mutant	Parent	Mutant
Phenol	500 mg/l	100	100	25	8
o-Chlorophenol	200 mg/l	100	100	52	26
m-Chlorophenol	200 mg/l	100	100	72	28
p-Chlorophenol	200 mg/l	100	100	96	33
2,4-Dichlorophenol	200 mg/l	100	100	96	34
2,5-Dichloropheno	200 mg/l	60	100	120	38
2,3,5-Trichlorophenol	200 mg/l	100	100	100	52
2,4,6-Trichlorophenol	200 mg/l	100	100	120	50
Pentachlorophenol	200 mg/l	7	26	120	120
o-Bromophenol	200 mg/l	100	100	85	14
m-Bromophenol	200 mg/l	51	100	96	25
p-Bromophenol	200 mg/l	87	100	84	22
2,4-Dibromophenol	200 mg/l	75	100	72	20
2,5-Dibromophenol	200 mg/l	58	100	120	35
2,4,6-Tribromophenol	200 mg/l	14	92	120	42

TABLE 4. DEGRADATION OF ALIPHATIC AMINES BY A MUTANT AEROBACTER SP. (a) 30°C

Compound	Conc	Degradation %		Time in Hours	
		Parent	Mutant	Parent	Mutant
Triethylamine	200 mg/l	100	100	28	11
N-Propylamine	200 mg/l	100	100	31	9
Di-N-Propylamine	200 mg/l	100	100	26	12
Tri-N-Propylamine	200 mg/l	100	100	30	10
N-Butylmanine	200 mg/l	100	100	22	7
N-Amylamine	200 mg/l	100	100	25	9
N-Hexylamine	200 mg/l	100	100	20	10
N-Dodecylamine	200 mg/l	100	100	18	5
N-Allylamine	200 mg/l	78	100	93	13
Di-N-Allylamine	200 mg/l	62	100	105	17
Tri-N-Allylamine	200 mg/l	47	100	120	22

TABLE 5. DEGRADATION OF ARYL AMINES BY A MUTANT AEROBACTER SP. (a) 30°C

Compound	Conc	Ring Disruption %		Time in Hours	
		Parent	Mutant	Parent	Mutant
Aniline	500 mg/l	100	100	54	12
o-Chloroaniline	500 mg/l	100	100	60	18
m-Chloroaniline	500 mg/l	100	100	68	16
p-Chloroaniline	500 mg/l	100	100	59	12
2,4,6-Trichloroaniline	500 mg/l	82	100	120	30
o-Toluidine	500 mg/l	100	100	64	6
m-Toluidine	500 mg/l	100	100	62	10
p-Toluidine	500 mg/l	100	100	48	3
o-Anisidine	500 mg/l	92	100	120	16
m-Anisidine	500 mg/l	80	100	120	24
p-Anisidine	500 mg/l	86	100	120	12
o-Dianisidine	500 mg/l	78	100	120	36

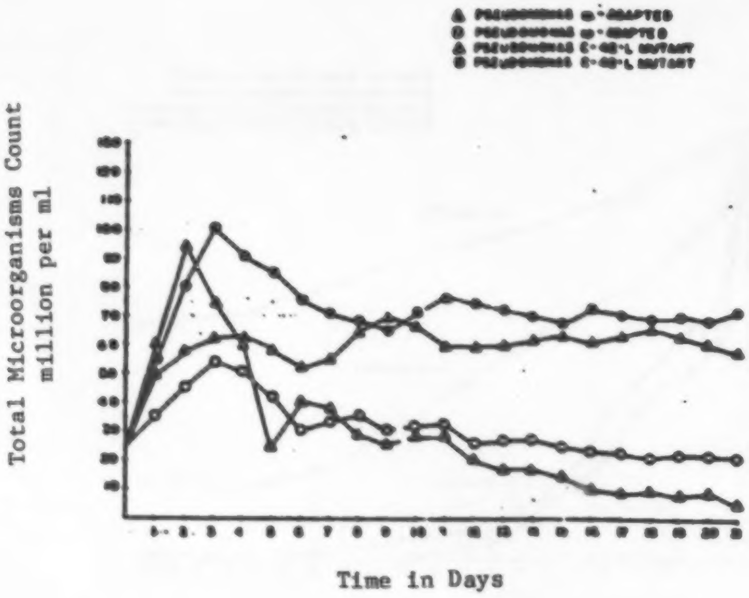

Figure 3. Growth of adapted and mutated Pseudomonas on ABS-LAS substrates in Worne media at 25°C.

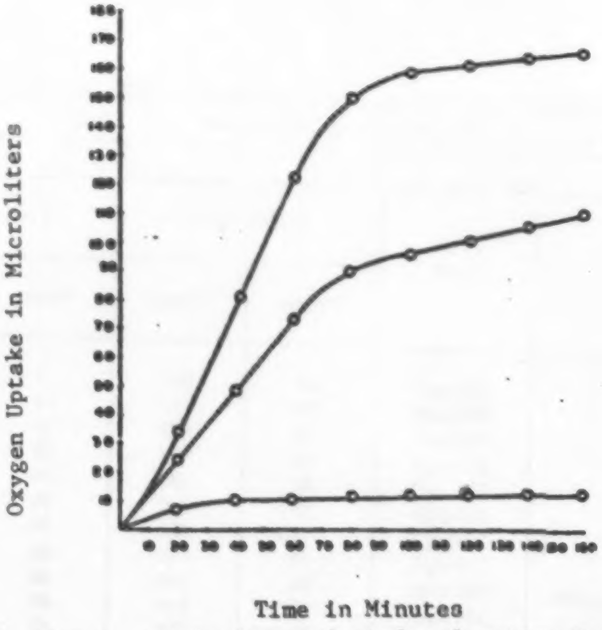

Figure 4. Oxygen uptake by unadapted and mutant Pseudomonas sp. in Worne media containing 0.01% solution of Sodium Alkyl Benzene Sulfonate at 25°C.

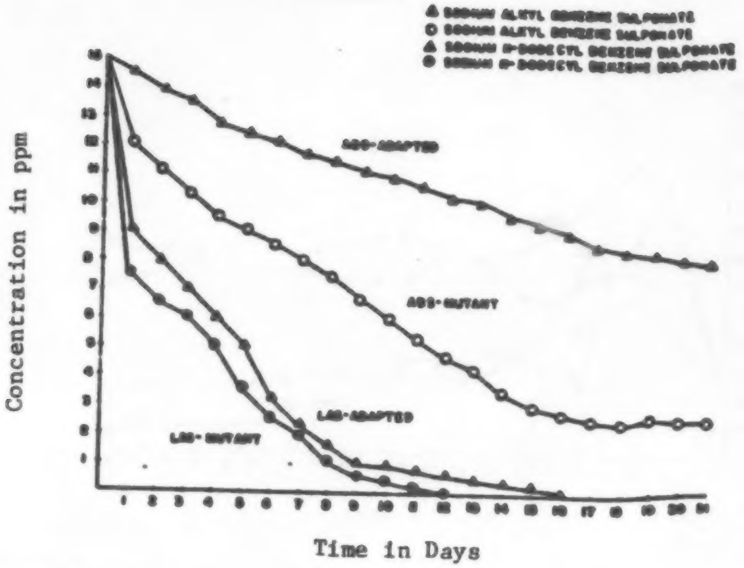

Figure 5. Biodegradation of ABS-LAS Substrates by Adapted and Mutated Pseudomonas sp. on Worne Media at 25°C

TABLE 6. BIODEGRADATION OF VARIOUS INORGANIC AND ORGANIC CYANIDES BY MUTANT MICROORGANISMS (a) 20°C

Compound	Conc	% Disruption		Time in Hours	
		Parent	Mutant	Parent	Mutant
Acetronile	500 mg/l	100	100	9	1.5
Butyronitrile	500 mg/l	100	100	13	4.0
Acrylonitrile	500 mg/l	84	100	24	4.0
Methacrylonitrile	500 mg/l	93	100	28	3.5
Phenylacetronile	500 mg/l	85	100	48	12.0
Phenylnitrile	500 mg/l	95	100	48	10.0
Phthalonitrile	500 mg/l	68-P *	100	48	15.5
Phenyl Isocyanate	500 mg/l	90	100	48	8.0
Dichlorophenyl Isocyanate	500 mg/l	92	100	48	8.0
NACN	250 mg/l	58-P *	100	48	1.0
HCN	250 mg/l	64-P *	100	48	0.5

* Poisoned

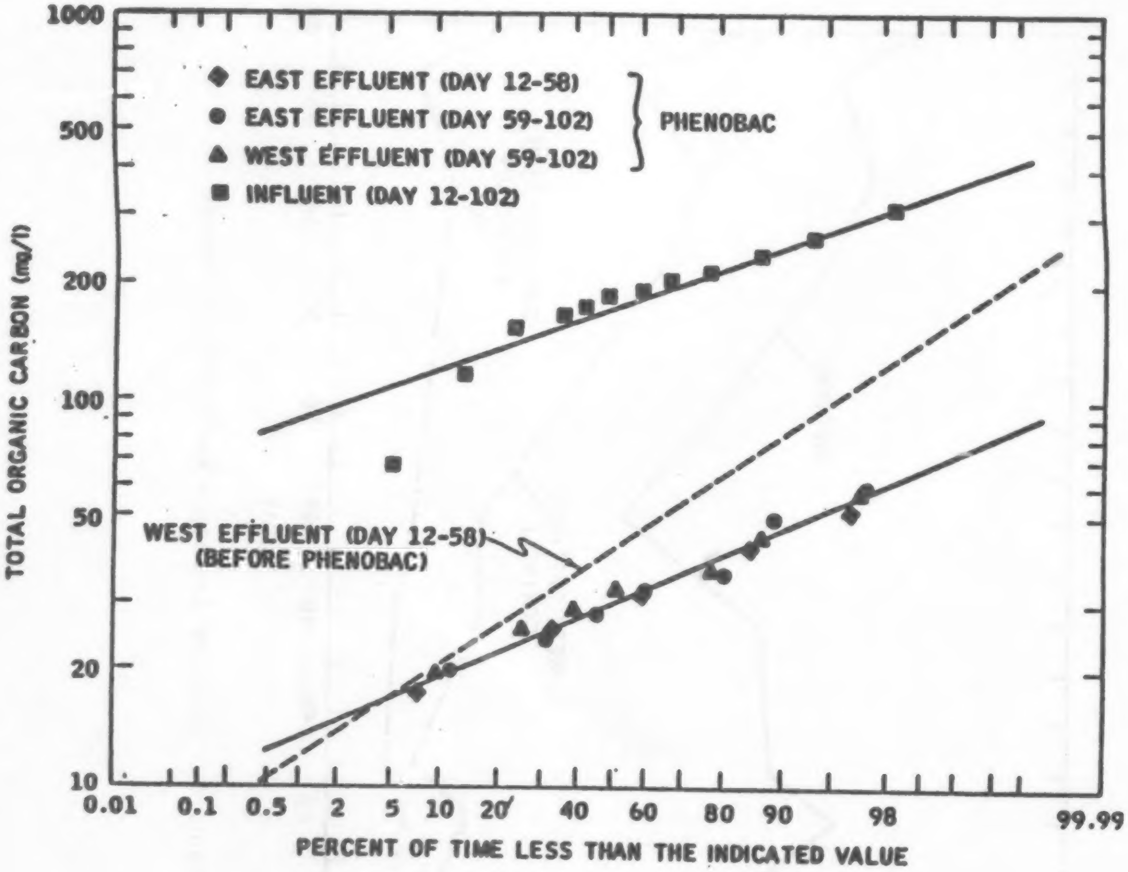

Figure 6. Activated Sludge Performance With Additive

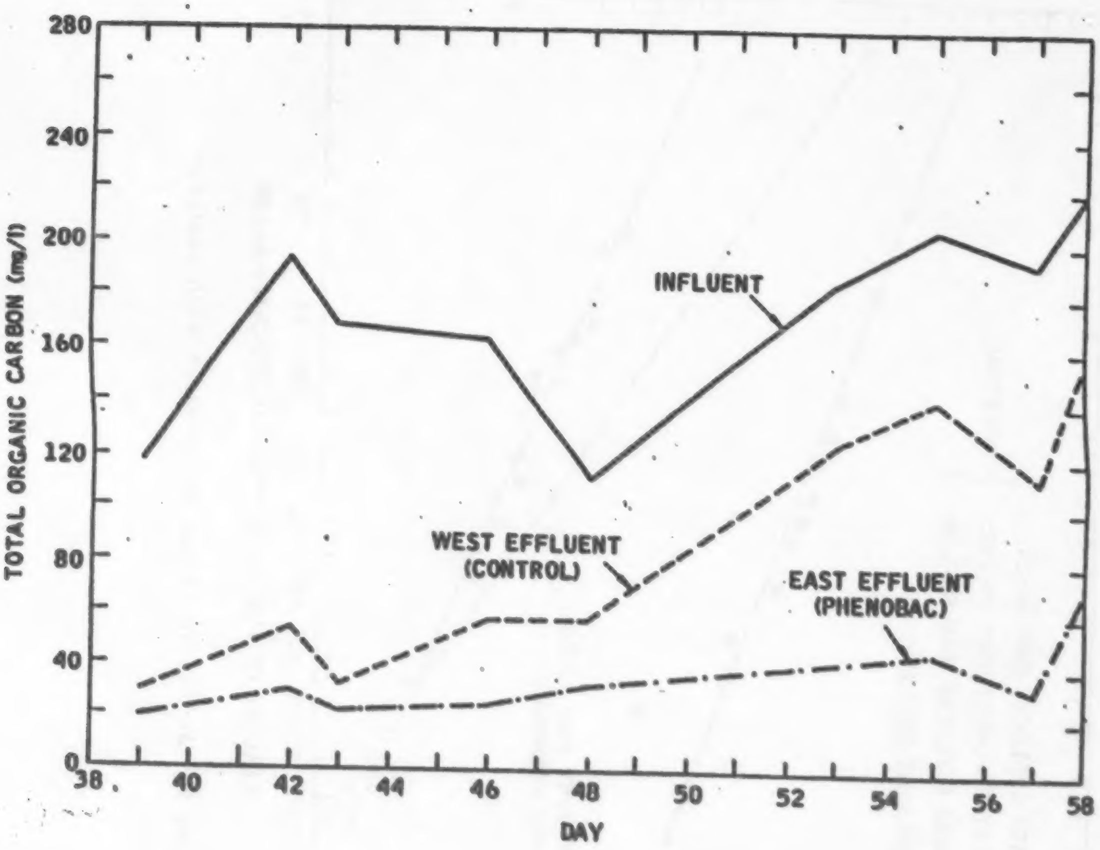

Figure 7. Additive Increases Resistance To Upsets

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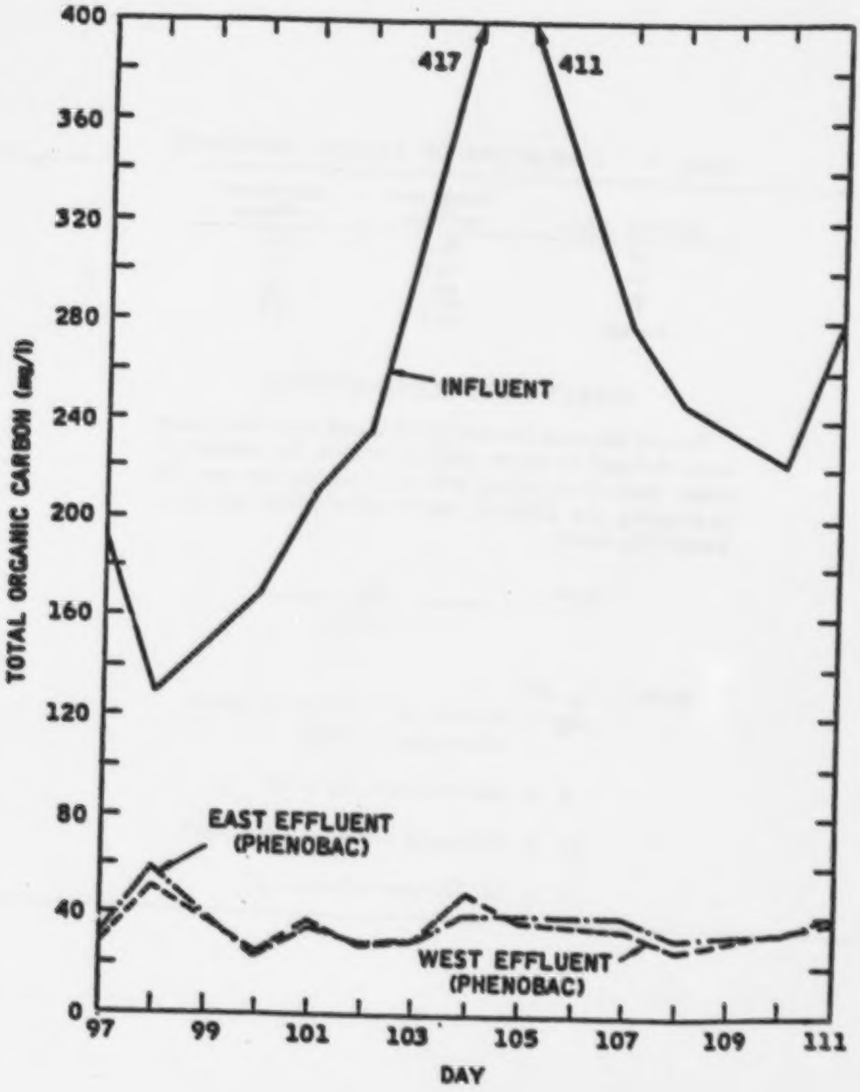


Figure 8. Increased shock resistance in treated units.

TABLE 7. COMPARISON OF KINETIC CONSTANTS

Percentile TOC	Treated unit coefficient	Control unit coefficient
10	32.6	12.6
50	53.8	6.5
90	42.6	3.9
Average	43.0	7.7

EFFECT ON PROCESS KINETICS

Process kinetics for the treated and untreated cases were defined to assess additive effects for conditions other than those which prevailed during the test. In evaluating the kinetics the following mathematical model was used:

$$S_e = \frac{S_o}{1 + KX\Theta_h}$$

where: S_o and S_e = influent and effluent substrate concentration, mg/l

K = rate constant, l/g—day

Θ_h = hydraulic retention time, days.

X = MLSS concentration, g/l

by Thibault and Tracy (37). Operation problems cited were solids-liquid separation in secondary clarification, a deterioration in effluent quality resulting from shock influent loads, and excessive adsorption of oil on the biological floc. The test program was conducted in two phases. The initial phase of the study was a three week trial of a parallel or side-by-side comparative analysis of the dual train activated sludge process while the second phase involved two consecutive fifty-day periods. The first fifty-day period prior to bacterial inoculation was used as a comparison with the performance during the second fifty-day segment.

Following one week of parallel examination, the trial portion of the activated sludge process receiving the special bacteria exhibited a difference in effluent quality. During the subsequent two weeks, it experienced a 21 percent improvement in effluent total oxygen demand (TOD) and a major reduction in floating solids. This phase of the study was terminated after 3 weeks because the activated sludge was operating at a temperature of 43°C.

The second phase of bacterial inoculation study was conducted using "before" and "after" performance data. Activated sludge effluent quality for TOD, BOD_5 (both total and soluble) and TSS was monitored during a 50-day period of bacterial augmentation. This performance period was then compared with the preceeding 50-day period which served as the control. Mean influent TOD quality to the activated sludge process during the control and inoculation periods was approximately the same, however, less influent variability occurred during the bacterial inoculation period. Comparison of effluent quality for the two periods indicated the following improvement during the inoculation period: TOD = 46 percent, BOD_5 = 73 percent; BOD_5 (soluble) = 59 percent, and TSS = 38 percent.

The authors used a mathematical model to estimate the kinetics during the control and inoculation periods. Their analysis indicated a greater than 2.5 increase in reaction rate attributed to the use of the mutant organisms during the inoculation period. Comparison of influent TOD, effluent TOD, BOD, TSS data before and after Phenobac application is shown in Figures 9, 10, 11 and 12, respectively. Improvement of treatment efficiency in a side-by-side demonstration, and improved degradaton of tertiary butanol with the use of Phenobac are illustrated respectively in Figures 13 and 14. Phenobac performance during acclimation period is shown in Figure 15.

Use of bioaugmentation products for improvement of waste removal reliability in wastewater treatment systems through enhancement of population dynamics and growth rate kinetics (23,32,38,39) has been described in the literature. It is the contention of the bioaugmentation technologists, that while it is true that the influent does influence the population dynamics of a wastewater treatment system by the very nature of its available nutrients and BOD strength, the selection process for microorganisms does not necessarily lead to an optimal microflora for the best assimilation rate of the pollutant loadings being applied. While "naturally" developed microflora can provide an adequate biological population for many waste streams, there are some waste streams beset with problems of bulking,

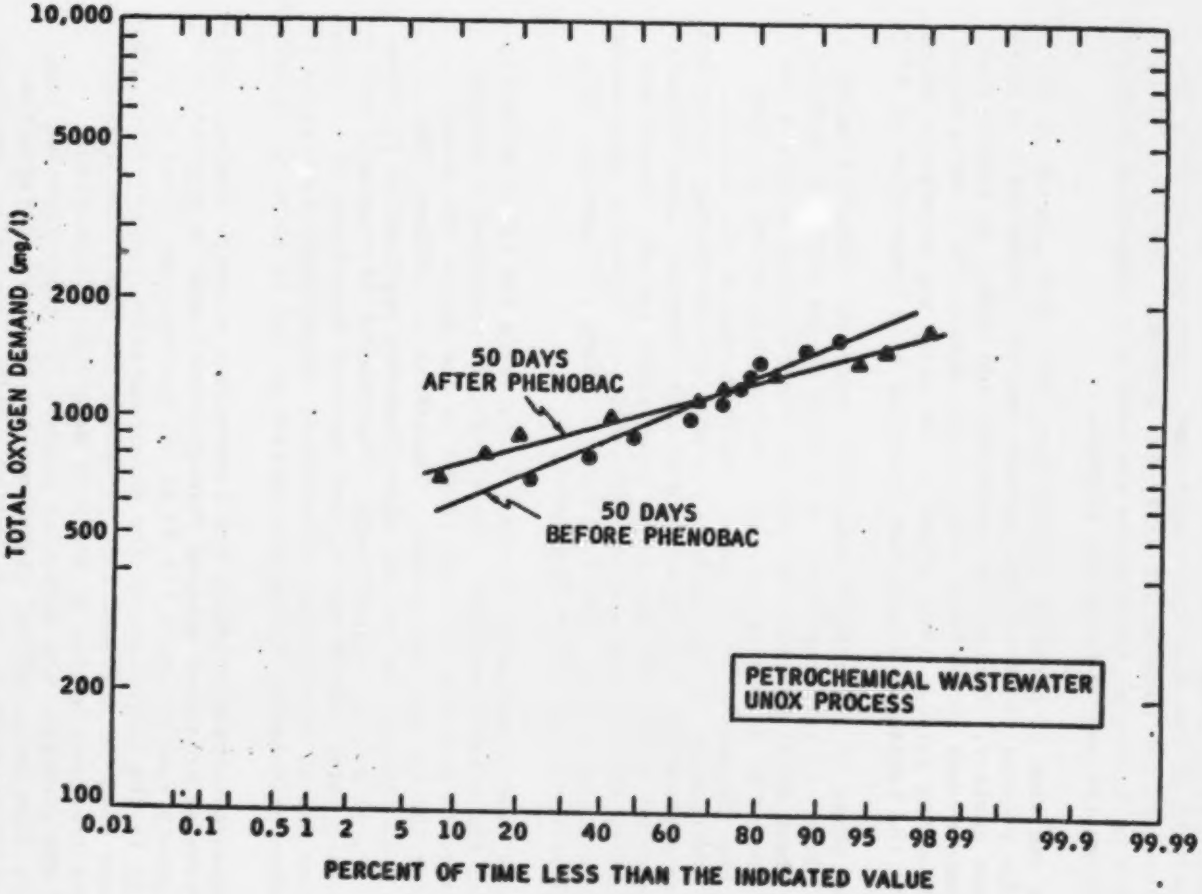


Figure 9. Comparison of Influent TOD Data Before and After Phenobac Application Reveals Similar Distributions

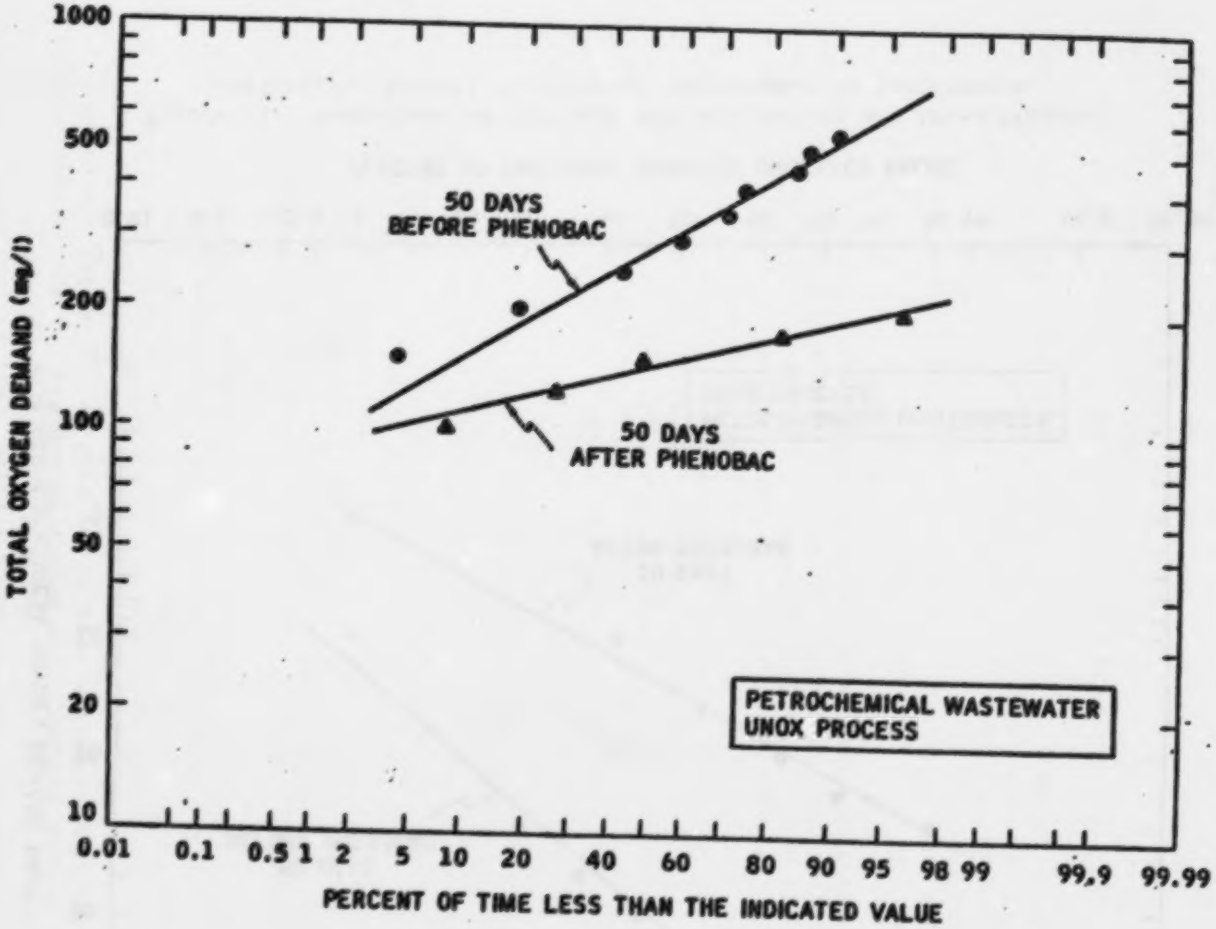


Figure 10. Comparison of Effluent TOD Data Before and After Phenobac Application Reveals Significant Improvement in Performance

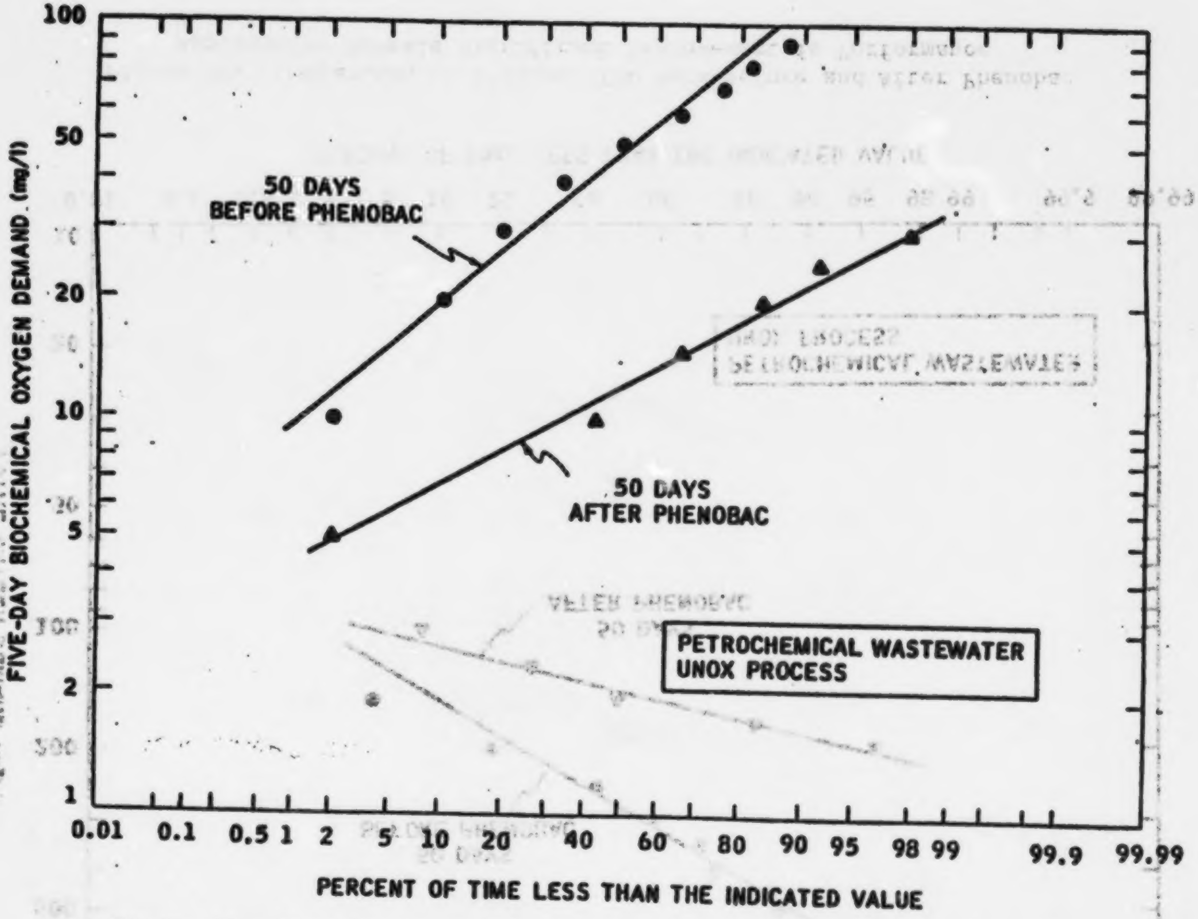


Figure 11. Comparison of Effluent BOD Data Before and After Phenobac Application Reveals Significant Improvement in Performance

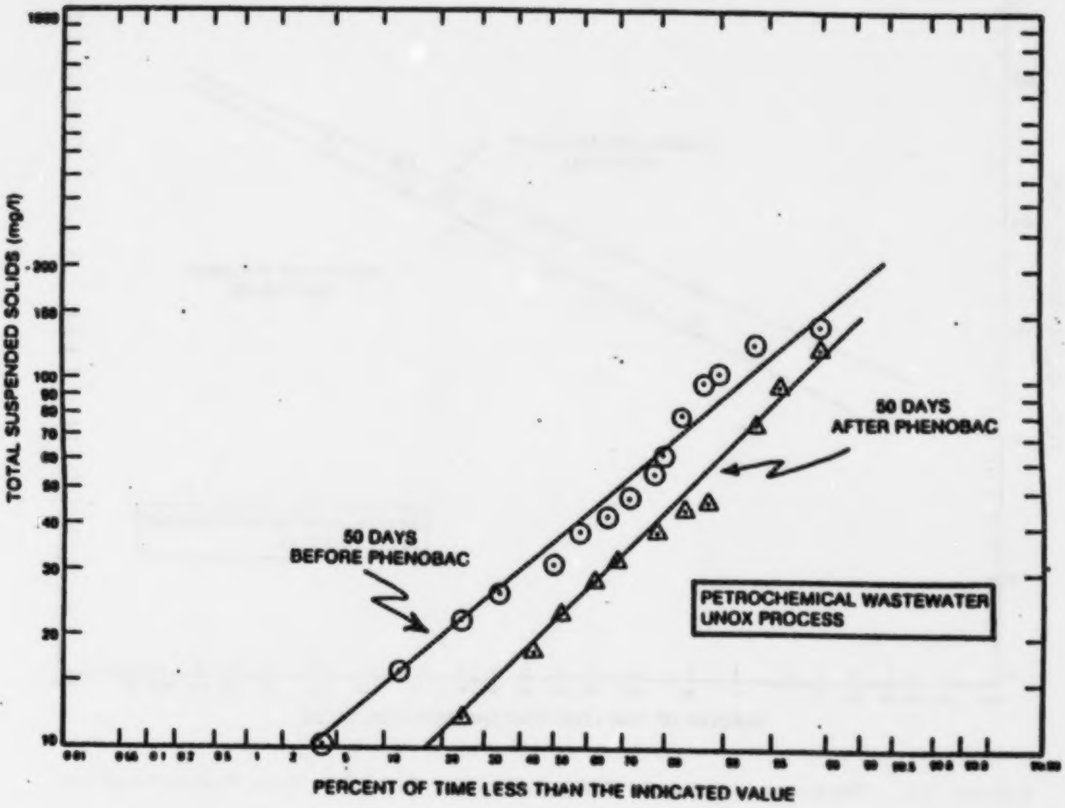


Figure 12. Comparison of Effluent TSS Data Before and After Phenobac Application Reveals Significant Improvement in Performance

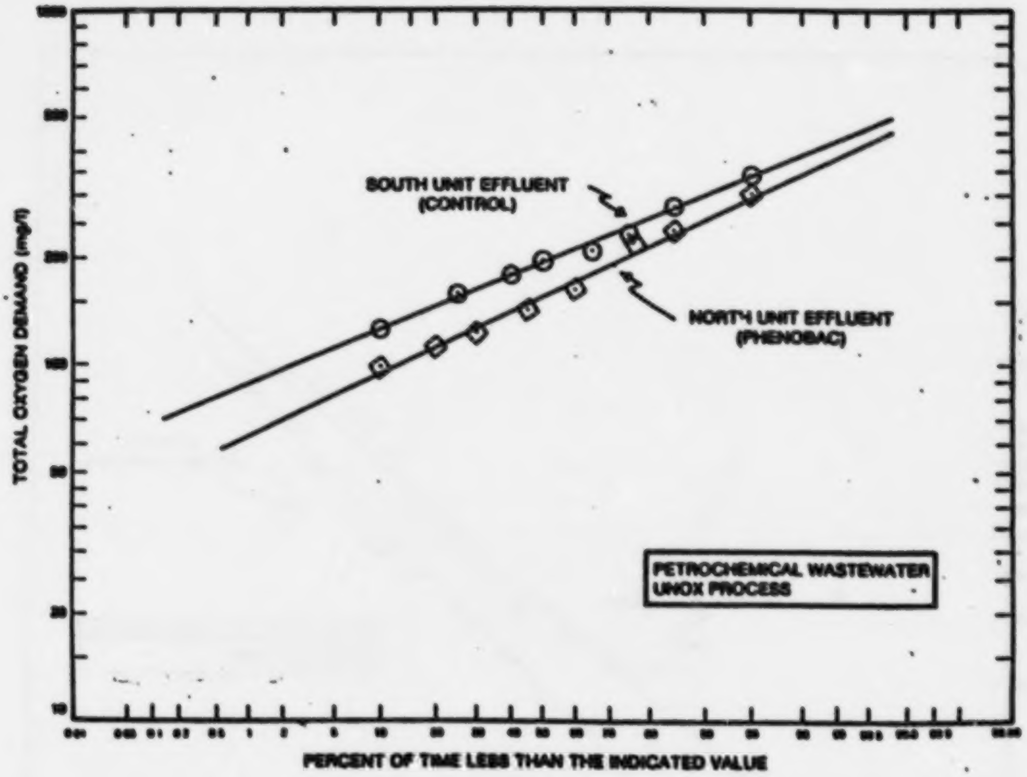


Figure 13. Phenobac Improves Efficiency in Side-by-Side Demonstration

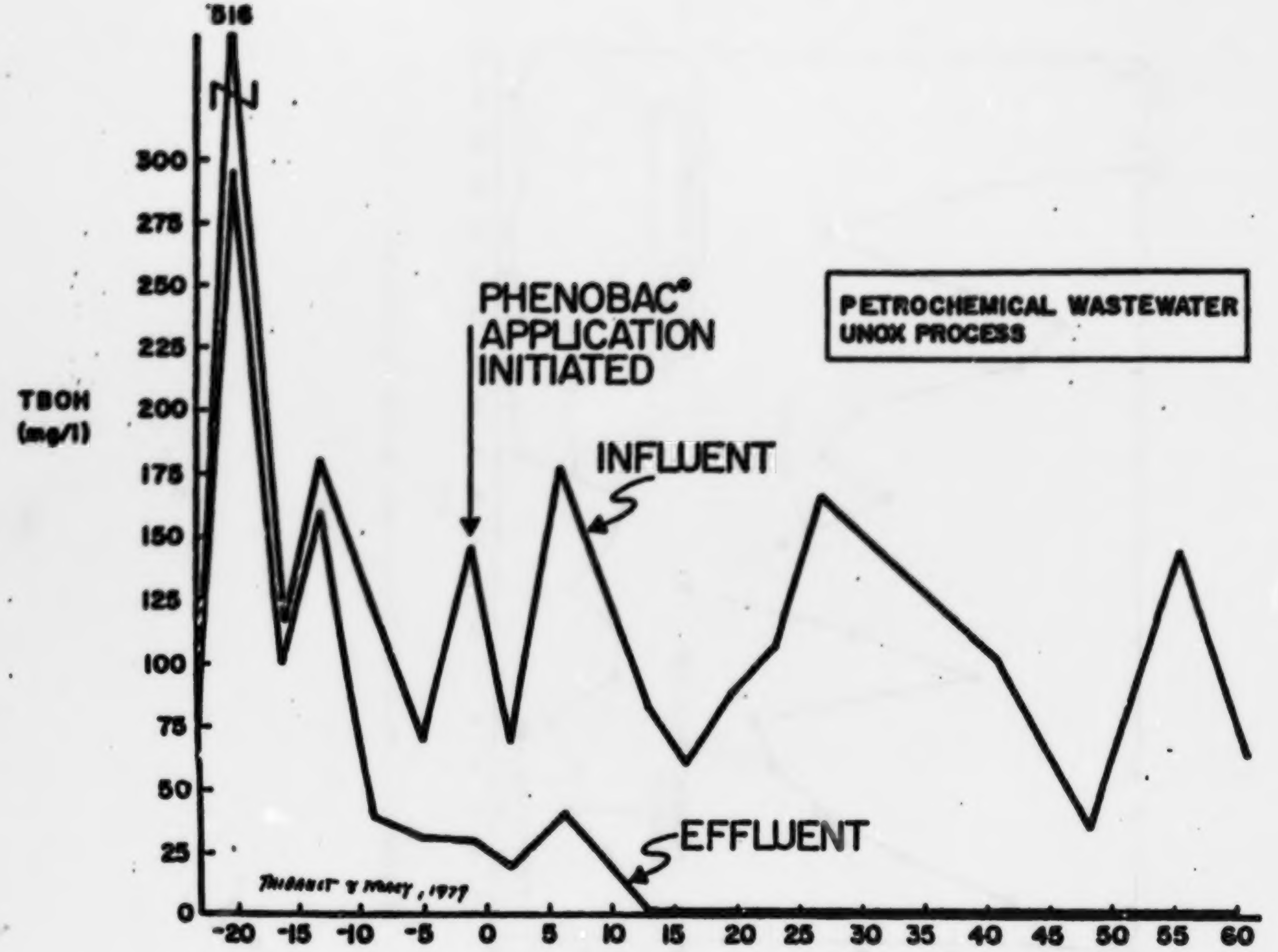


Figure 14. Phenobac Improves Degradation of Tertiary Butanol

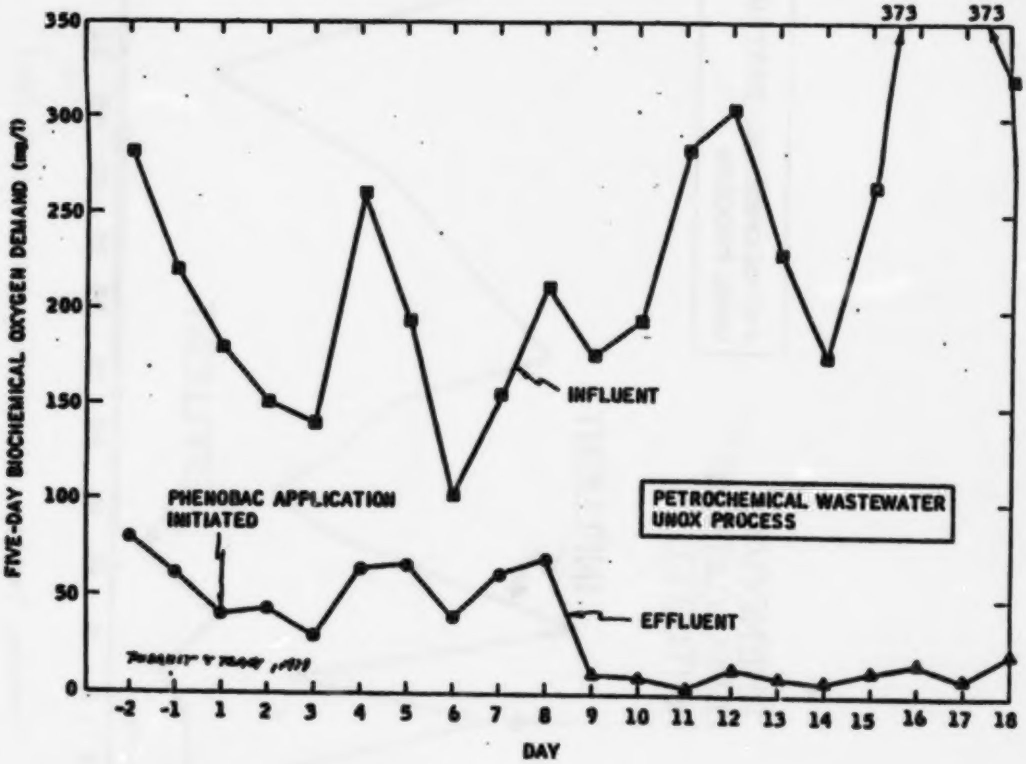


Figure 15. Phenobac Performance During Acclimation Period

deficiency in settling solids and an excessive loss of suspended solids in the discharge. The naturally selected microbiota do not provide a reliable waste removal capability for the wastewaters. Researchers in biotechnology maintain that it is possible to influence the population dynamics of various waste treatment systems (i.e. dairy waste) microflora by altering their microbial population and improving removal performance through bioaugmentation.

Chambers (23) maintains, that to optimize a biological waste treatment process, several growth factors must be designed into the system to achieve the desired water purification. These factors are: to provide and maintain a physiochemical environment that minimizes suppressive factors on the waste assimilation process and growth of the biomass; to supply adequate aeration and mixing to meet the respiratory needs of the biomass microflora and to assure stabilization of the organic waste constituents; to promote the establishment of desirable types and quantity of microorganisms to assimilate the pollutants present in the waste stream; and to control the balance between the BOD being applied and the quantity of biomass needed to reduce this loading to a desirable level in the final discharge. However, the key to the successful treatment of a given waste stream is the capability of the biomass to assimilate the waste, convert this waste into cells and suspended solids and form a floc particle that will settle. In accomplishing the above, the control of the biomass activity is dependent upon detention time, control of biomass solids and the maintenance of a desirable quantity ratio between nutrient loading and biomass solids in the system. According to Chambers (23) microbial constituents of biomass solids are critical to the nutrient uptake and new solids yield rate, based on growth kinetics of biomass. The higher the growth rate the better the efficiency rate will be for waste removal.

Chambers further indicates that bioaugmentation permits the establishment of selected microbiota which compete more effectively for substrate and are more tolerant to vascillating growth conditions. Eventually, the selected bacteria gain population dominance, waste assimilation properties are improved, and waste removal performance is more reliable. In essence, the population dynamics is altered through the use of the bioaugmentation technique.

McDowell and Zitrides (32) have demonstrated Monod's concept of cell growth response to substrate concentration by using adapted mutant strains of microbiota specifically selected to assimilate petroleum refinery wastes. Their observation supported the Monod concept that it is the total amount of enzyme produced by a given microorganism which dictates the growth response of the cell. Additionally, the Michaelis-Menton constant, Ks (Monod) for the limiting substrate can have a direct influence of the microorganism's ability to compete for available substrate. That is, a microbial cell which has a lower limiting substrate requirement needed to drive essential metabolic reaction will have a decided advantage over other competing cells. This competitive advantage assists the microbial cell in establishing itself in the waste assimilation environment and certainly influences the population dynamics.

According to McDowell and Zitrides (32) competition for the same food source always favors the biological cell that has the lower Ks constant. Decided advantages are a lower limiting substrate requirement, a quicker growth response and shorter cell generation times. For a nonrestrictive substrate concentration, all competing microbiota will grow and multiply but at different growth rates. As the substrate concentration becomes growth limiting, cells with higher Ks requirements will begin to cease being competitive. At that point, microbial cells with the lower Ks substrate requirements will begin to dominate.

The success of the bioaugmentation program lies in the correct selection of bacteria to assimilate a given waste stream. Those bacteria introduced into the waste assimilation environment must be able to actively compete for available food and eventually gain population dominance. Usually this competitiveness is due to the cell's lower Ks (Monod) substrate requirements, its shorter generation time, and an ability to respond rapidly to a favorable growth environment.

According to Chambers (23) and McDowell and Zitrides (32) benefits that should be realized through the use of boiaugmentation are: decreased sludge solids yields due to a more efficient destruction of colloidal material; rapid establishment at start-up or restoration after chlorination of returning sludge of the biological activity in the wastewater treatment process; improved floc formation and settling; and increased waste assimilation rates and versatility in substrate uptake.

Enhancement of dairy waste effluent treatment with the use of specifically adapted/mutant microbial supplementation (22,23,40-45) has been reported in literature. Chambers (23) described the application of bioaugmentation to an aerated stabilization basin (ASB) system and two activated sludge processes operating in the dairy industry. The activated sludges exhibited poor sludge settling characteristics while the ASB process produced a dispersed biological floc. Poor floc formation associated with dominant populations of fungal and filamentous bacterial species, resulted in less than desired levels for BOD and suspended solids being discharged by all three systems. Accordingly, the "normal" biological mass was supplemented with specific bacterial cultures. One source of bacteria, culture of Pseudomonas species was used to routinely inoculate one of the activated sludge processes. The second activated sludge system and the ASB process were augmented with a commercially available bacteria. Following the addition of the special microbiota, the activated sludges exhibited improved settling, compaction, and a decline in filamentous organisms. This response allowed a greater concentration of biological solids in the aeration process reducing the food-to-mass (F/M) ratio and decreasing the sludge production. Effluent quality for BOD_5 and suspended solids also improved following bioaugmentation. The bioaugmentation of the ASB produced results similar to the activated sludge inoculation program. A general improvement in effluent quality was experienced.

Reported experience with bacterial augmentation of biotreatment systems in the pulp and paper industry (46-51) includes trade journal articles, reports presented at technical meetings and scientific journals. Blosser (47) presented the source of industry effluents by manufacturing category where bacterial supplementation of biological waste treatment systems has been conducted. The Kraft category represents the majority of experience followed by mechanical pulping and paper production categories. Wastewater treatment at various mill locations represents a rather even distribution between the activated sludge (AST) and aerated stabilization basin (ASB) processes. With the exception of one mill location, pulp and paper industry experience with bacterial supplementation reflects the "before" and "after" approach to product evaluation, in that historical data provided the basis for comparison. This, according to Blosser (47) reflects the lack of opportunity within the physical design of the treatment systems to provide the control feature necessary for conducting a parallel examination.

Numerous studies documented in the literature report on the successful application of bioaugmentation products in the improvement of various aspects of aerobic wastewater treatment (4, 7-9, 32, 52-64). Similarly, improvements in the anaerobic sludge digestion and enhancement of anaerobic treatment (65-69) with the use of biocatalytic additives have been reported. Literature abounds with references regarding the implementation of microbial supplements in waste water systems for the improvement of sludge digestion (70-74); sludge management (75-77); sludge settling improvement (78; sludge filtration (79); and nitrification (80,81).

Many studies have also shown the useful application of enzyme preparations as biocatalytic additives (79, 82-91) in the waste treatment processes; in the treatment of primary sewage sludge; for the improvement of biosludge filtration; in the pretreatment system of industrial wastes and in treatment of specific organic toxic compounds; as well as in the food industry waste processing.

II. Research Results and Critiques by Critics of the Bioaugmentation Approach

Review of the studies of bacterial augmentation of biological treatment in the literature indicate that the majority of experience has resulted from examination of performance data from full scale processes comparing the trial period with before-and-after performance data. In those cases where the use of parallel treatment trains have been cited, no reference has been made to characterizing the process to determine what differences may exist between parallel treatment units. In addition, failure to identify process conditions in the trial and control systems during the course of the studies limits the utility of the data generated.

The literature also contains reports on a number of laboratory studies where bioaugmentation has been evaluated. Some researchers who criticize the bioaugmentation technology laboratory research studies, argue that although laboratory scale activated sludge studies provide a desired degree of control, they generally operate under dissolved oxygen levels and hydraulic clarification loadings far removed from conditions existing in full scale application of the process. In addition, it is indicated that such studies do not normally incorporate the variability of influent conditions to which a full scale process must respond.

Blosser (47) summarizes experience with bacterial augmentation of biological treatment process to improve performance in the treatment of pulp and paper industry wastes and reports on supplemented bacteria inoculations of a full scale activated sludge process treating a bleached kraft mill effluent. Pulp and paper industry experience with bacterial supplementation has predominantly resulted from full scale application using the before-and-after assessment approach and a number of such situations have been identified.

Blosser (47) reports a 150-day study conducted at a bleached kraft mill to examine bacterial augmentation of its activated sludge process. Specific goals assigned to the study were: improvement in effuent BOD_5 and suspended solids, with a greater emphasis on effluent suspended solids quality; an accelerated rate of recovery of the activated sludge from a process upset; reduction of biological sludge generation rate; and sustaining or improving activated sludge settleability and thickening properties.

The response of the control and trial (augmented) activated sludge processes at this specific bleached kraft mill have resulted in the following observations:

- The BOD_5 quality in the treated discharge from the activated sludge process was neither improved nor deteriorated by the use of bacterial augmentation.
- The data collected following augmentation trial indicated that the differences in suspended solids discharged from the trial and control portions of the treatment system were in effect, typical of system performance and not necessarily the result of bacterial supplementation.
- Activated sludge settling velocity and thickening potential were not influenced by bacterial augmentation.
- A reduction in biological sludge production, of approximately 0.10 lb TSS produced per lb of BOD_5 removed, observed in the trial activated sludge unit when compared to the control, was shown to be an inherent aspect in the performance of the dual train activated sludge process and could not necessarily be attributed to the use of the specialized bacteria.

Quasim and Stinehelfer (39) described the use of control and trial laboratory activated sludge units to determine the impact and performance of a special bacterial product. Batch reactors were used to select the optimum dosage of the freeze-dried microorganisms, based upon the maximum reduction of O_2 demanding substrate in a domestic wastewater. Evaluation of the bacterial additive was conducted in 2 identical continuous flow activated sludge units operating under the same conditions.

The utility of bioaugmentation was evaluated by two techniques. One was a statistical analysis of effluent BOD_5 quality for the dosed and control units, while the second approach compared the biological kinetic coefficients observed in the 2 units. The kinetic coefficients compared, were: biological sludge yield (Y), maximum rate of bio-oxidation (k), microorganisms decay (k_d) and the half velocity constant (Ks). Statistical analysis of the effluent quality indicated no significant difference between the control and dosed activated sludge units.

The authors concluded that addition of the special bacterial product had no effect on substrate utilization, k, and half velocity Ks coefficients. The dosed unit did exhibit modest differences in sludge yield and sludge decay coefficients, being slightly greater in the former and slightly less in the latter. The effect of the kinetic coefficients in the control and trial units was estimated using a Monod mathematical model. The model projected that the activated sludge unit receiving the special bacterial culture would produce a slightly lower BOD_5 concentration than the control unit, depending upon the sludge age of the process. There was little effect of the product on the overall performance of a well operated activated sludge plant. Perhaps the product may have some benefit in those plants that are already overloaded and are operating at poor organic removal efficiencies.

While most of the product manufacturers talk about the successes they have had with their products, little has been published about testing methodology. The intent of Quasim and Stinehelfer (39) studies was to present a methodology for evaluating such products. The procedure presented in the report provides a systematic approach in evaluation of bacterial culture products and is a widely used technique in developing design parameters for industrial and joint industrial-municipal wastewater treatment facilities. Because the kinetic coefficients developed for bench scale reactors are so valuable in the design of treatment plants, it is felt by the authors that the same procedure can be used to evaluate the performance of bacterial culture products for special applications such as the effect of these products on biological treatment plants under different operating conditions.

In his assessment on the use of enzymes and biocatalytic additives for wastewater treatment process, Young (91) maintains that adding commercially available enzyme products to both aerobic and anaerobic systems might not cause a dramatic change in the system's performance, since a full complement of different enzymes is generally needed to mediate the many complex series of biochemical reactions, beginning with the change of the parent substrate, going through many intermediate steps and finalizing in the end product. Such full complement composition of enzymes in any of the commercial bioaugmentation products is neither available nor economically feasible.

The exact composition of commercially available biocatalytic additives is often unavailable. As either dried bacterial solids, by-products of bacterial fermentation reactions or a combination of microbial cells and their fermentation products, these additives are not pure enzymes and possibly could contain about 1% enzyme by weight even though no separation or purification step is involved. According to Young and others, it is difficult to see how the small dosage of dried cultures recommended by the suppliers can overcome the effect of the large amounts of bacteria already present in the wastewater treatment process. If the environment was favorable for their existence, they would already be there in large quantities.

Again, according to the critics, it is difficult to see how a commercial biocatalytic formulation containing by-products of microbial fermentation reactions, even if used at full strength, can cause a dramatic increase in the breakdown of organic materials, and how these preparations can help decompose materials which can not be decomposed by the bacteria which have been in constant contact with the waste materials.

In addition, the use of the bioaugmentation products at quantities needed and for the duration of time recommended by suppliers to enhance a specific treatment of waste, may be quite cost prohibitive. In a number of cases, where enzymes have aided treatment, it is the feeling of the critics that there is no way to tell if the enzyme would have been more effective, if some of the operating process control strategies had been modified, before addition of enzyme, to effect a better treatment system.

Biocatalytic additives frequently have been misapplied, according to critics of bioaugmentation approach. The critics point out that it is almost impossible for biocatalytic agents added in quantities normally recommended by suppliers to improve the performance or capacity of properly designed and operating treatment plants, including anaerobic digesters. When fats, proteins and carbohydrates accumulate to such an extent that problems occur, biocatalytic agents may be beneficial if they can be justified economically.

According to Young (91) a number of significant recommendations were made by the Special Committee on Enzyme and Biocatalytic Additives of the Water Pollution Control Federation. These recommendations are summarized as follows:

- Biocatalytic additives should not be purchased until after they have performed as claimed in accordance with a written guarantee.

- Advertising literature should be rewritten clearly and correctly so as not to misrepresent the merits of the product.

- Distributors should deal directly with engineers, superintendents or operators rather than attempt to influence mayors, councilmen, or members of boards of public works.

- Before the additive is considered, it should be tested so that fundamental and reliable data can be obtained.

- When digestion problems develop, major efforts should focus on proven methods of analysis and recovery.

These recommendations have been published in the Water Pollution Control Federation Manual of Practice No. 16 on Anaerobic Sludge Digestion. The Manual concludes that biocatalytic additives cannot be added economically in effective quantities.

Rittman (92) in his discussion of the relationship between genetic-control and process control strategies in municipal wastewater treatment points to the following problems in current biological processes: poor reliability of treatment and poor performance; characteristics of sewage input: high volumes, variable flow rates, low and variable constituent concentrations and sudden shock loads caused by precipitation or industrial discharges; and high cost of treatment. He outlines the following approaches to enhance treatment performance and enhancement of reliability and/or economics of biological treatment: make a process related improvement; apply an existing process in a new situation; select novel microorganisms; and genetically engineer appropriate microbiota to have a new desired function or trait.

Rittman stresses the need for coordinating the process control with genetic engineering control. He maintains that genetically controlled microorganisms must continually proliferate and must be able to grow and out-compete its rival competing strains of microbiota for the various substrates. He states that addition of exogenous biomass at the tons/day rate would be expensive if not practically impossible. Initial and maintenance additions of mutant bacteria are insignificant compared to the total production of biomass. Only if mutant bacteria can grow and out-compete the indigenous strains would bioaugmentation be valuable and if such growth occurs, maintenance additions would then be unnecessary. Rittman also points out that causes of process control problems are never identified in bioaugmentation literature.

EVALUATION STUDIES OF BIOAUGMENTATION PRODUCTS

Bioaugmentation products are manufactured by an increasing number of industries to enhance specific wastewater treatment and sludge handling capabilities. With the increasing use of these products, the Environmental Protection Agency (EPA) recently initiated a program to evaluate the bioaugmentation technology and to assess the legitimacy and effectiveness of the manufactured biocatalytic products through the use of standard testing protocols. The goals of this program are threefold: first, to place the use and characterization of these types of products on a scientific basis; second, to arrive at consensus-testing standards for label verification/package contents and for performance of these bioaugmentation products; third, to assess capability of the bioaugmentation technology through the use of the standard testing protocols on biocatalytic products. Accordingly, the National Sanitation Foundation (NSF) in Ann Arbor, Michigan was funded by the EPA under the Cooperative Agreement No. CR-811884 to meet these goals.

The specific objectives of the Agreement are: to review and categorize the current array of bioaugmentation products by type of generic category and by application; to develop and validate the standard procedures to insure that package contents are consistent with package labeling; to develop and validate standardized test procedures to evaluate product performance against manufacturer claims; to bring product manufacturers together to launch the development of a national voluntary concensus standard for the bioaugmentation products; and, to implement an ongoing, self-supporting program of independent testing and evaluation based on these standards and the developed test protocols with the aim of achieving a product certification system.

The project was divided into four general Phases. A completed Phase I consisted of gathering information on bioaugmentation products and test methods and the formation of a Technical Advisory Committee and an Industry Committee. Both committees were given responsibility to review and comment on data summaries and reports. The Technical Advisory Committee was assigned responsibility for test protocol development. The Industry Committee was asked to draw on its experience with test methodologies to assist in protocol refinement. The ongoing Phase II includes the development of detailed protocols, product testing, protocol refinement and validation and review of results. In the future, Phase III will disseminate results and develop the final reports. Phase IV, which does not rely on project funds, will develop a national voluntary consensus standard for bioaugmentation products.

In Phase I, the Technical Advisory Committee established, (Table 8 provides a list of committee members) five major categories of potential product applications. These were: aerobic treatment; anaerobic treatment; sludge handling and digestion; the in-sewer treatment; and specific

TABLE 8. BIOAUGMENTATION ADVISORY COMMITTEE

	Phone Number		
Dr. James V. Chambers Smith Hall 101 D Dept. of Food Sciences Purdue University West Lafayette, IN 47907	317-494-8279	Mr. Howard Selover Chief Operator Training Unit Community Asistance Div. Department of Natural Resources Lansing, MI 48909	517-373-0397
Mr. Donald Kerr (observer) US Department of Agriculture Food Ingredient Assesment Div. Washington, DC 20250	202-447-7680	Dr. Jospeh Trauring Pollu-Tech Inc. PO Box 77 Chalfont, PA 18914	215-822-8123
Mr. Eugene DeMichele Water Pollution Control Federation 2626 Pennsylvania Ave., NW Washington, DC 20037	202-337-2500	Dr. Leon Weinberger Peer Consultants Inc. 1160 Rothville Pike Suite 202 Rockville, MD 20852	301-340-7990
Mr. William Hill V.P. Operations and Maintenance Camp Dresser & McKee Inc. One Center Plaza Boston, MA 02108	617-742-5151	Dr. David R. Zenz Metropolitan Sanitary Dist. of Greater Chicago R & D Laboratory 5915 W. 39 St. Cicero, IL 60605	312-780-4060
Dr. Henryk Melcer, Chief Biological Treatment Environmental Protection Service Canada Centre for Inland Waters Burlington, Ontario L7R-4A6	416-637-4546	Dr. Joe Kang McNamee, Porter and Seeley 3131 South State Street Ann Arbor, Michigan 48104	313-665-6000

substrate removal. Top priorities (Table 9) included all categories except in-sewer treatment. The NSF Staff and the Technical Advisory Committee also established a Bioaugmentation Industry list. This list includes those identified by NSF as being involved and/or interested in the bioaugmentation industry: manufacturers, reformulators, vendors and consultant/users. This list is not inclusive and does not contribute an endorsement by the EPA or NSF of the companies listed.

Also in Phase I, at the first Bioaugmentation Industry Meeting, held on November 27, 1984, the industry recognized the value of a certification program and was fully appreciative of the EPA's attempt to improve the credibility of the bioaugmentation technology and the biocatalytic products. They also recognized that the existing assays were not uniform across the industry for specific bioaugmentation activities. They became aware of a need for the industry to financially support field testing, after the completion of the NSF laboratory investigation, and eventually to develop a certification process. After meeting with the Industry Committee, the Technical Advisory Committee of the Bioaugmentation Products Evaluation Studies, working closely with the NSF research team and the EPA project officer, began the development of the label verification and performance testing protocols. At a second Bioaugmentation Industry Meeting on March 1, 1985, attended by a cross-section of producers and suppliers as well as technical representatives of the bioaugmentation industry, the NSF research team and members of the Technical Advisory Committee, a general consensus and agreement was reached on the proposed draft label verification and efficacy testing protocols for the project. It was also agreed that the voluntarily obtained bioaugmentation products would be tested by the NSF. A separate program to develop policies and procedures for national voluntary consensus standards was initiated concurrently with the EPA project.

After the Second Bioaugmentation Industry Meeting, Dr. Robert Wolfe, Professor in the Biostatistics Department, University of Michigan, School of Public Health, developed a statistically sound experimental design for the Label Verification and Performance Protocols. He is also performing statistical analysis of the generated data throughout the evaluation study at National Sanitation Foundation. With the appropriate quality control, Dr. Wolfe's statistical analysis experimental design for the continuous flow phase of the performance (efficacy) protocol is based on the Latin Square approach. The Latin Square statistical experimental design approach, reviewed by the U.S. EPA statistical team, is very appropriate for the use in performance-testing of biocatalysts in both the synthetic and raw wastewater media in either sequential or concurrent experimental runs.

DESCRIPTION OF TESTING PROTOCOLS FOR EVALUATING BIOAUGMENTATION PRODUCTS

Protocol testing was divided into three areas of effort: label verification/product classification, bench scale performance (efficacy) testing, and field performance testing. A flow diagram for the overall approach involving the protocol development process is presented in Figure 16.

TABLE 9. MAJOR CATEGORIES OF POTENTIAL BIOAUGMENTATION PRODUCT APPLICATION

Four major categories of potential product applications were defined. These were: in-sewer treatment, aerobic treatment, anaerobic treatment, and sludge handling. The committee chose aerobic treatment and sludge handling as the two categories requiring the most attention. The major categories and their applications, as prioritized by the group, are outlined below.

AEROBIC TREATMENT	SLUDGE HANDLING	ANAEROBIC TREATMENT	IN-SEWER TREATMENT
1. Reduce sludge production	1. Sidestream improvement	1. Digestion improvement	1. Odor control
2. Improve efficiency	2. Thickening/ dewatering	2. Septic tanks	2. Cleaning
3. Improve clarification	3. Composting	3. Improve mainstream treatment	3. Corrosion control
4. Specific sustrate removal (including nitrification and denitrification)			4. In-line treatment
5. Improve startup time			

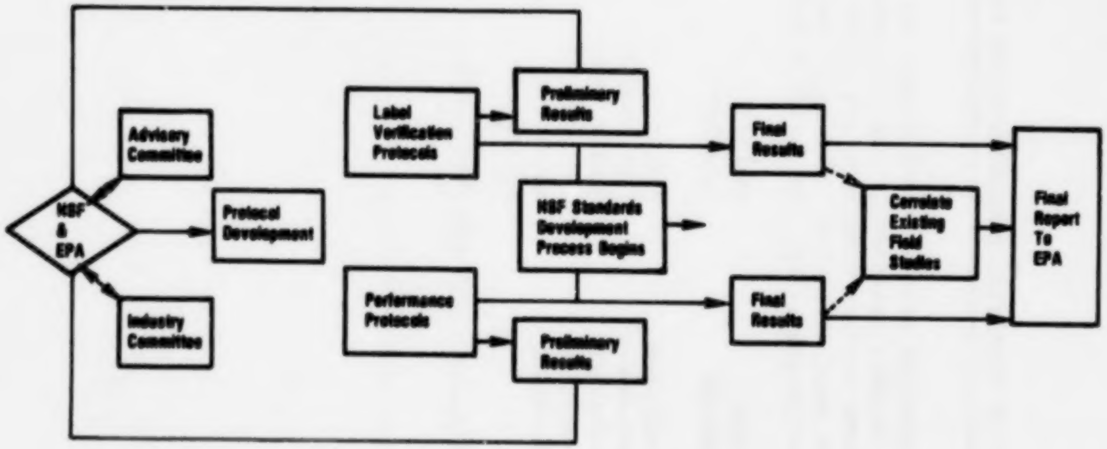

NSF & EPA
Advisory Committee
Protocol Development
Industry Committee
Label Verification Protocols
Performance Protocols
Preliminary Results
NSF Standards Development Process Begins
Preliminary Results
Final Results
Final Results
Correlate Existing Field Studies
Final Report To EPA

Figure 16. Bioaugmentation Study Design.

PRODUCT CLASSIFICATION AND LABEL VERIFICATION PROTOCOL

The objectives of the Label Verification/Product Classification portion of the bioaugmentation products evaluation study are listed as follows:

- Develop and test reliable protocols to determine the potency of bioaugmentation products with potency measured in terms of number of viable organisms grown on eight different agar/supplement combinations, enzyme activity, and/or whole cell activity (e.g. specific substrate reduction, oxygen uptake, TOC removal).
- Determine the consistency of products submitted for evaluation with consistency measured as the reproducibility of product potency determined on replicate subsamples of different lots of samples secured from the same industry at different times.
- Enumerate and identify the active microbiota and correlate the findings with information provided by industry.
- Develop an overview of the bioaugmentation products as measured by the range of potency of the bioaugmentation products.

The Product Classification and Label Verification Protocol includes a procedure for plate counts (aerobic and anaerobic) using Standard Methods agar media and Reasoner agar media (R2A) to quantify, characterize and identify viable microbiota in all products. Composition of R2A medium is outlined in Table 10. The protocol also includes total plate counts, supplemented with specific substrates to detect enzyme activity (protease, lipase, amylase, etc.) and to observe specific substrate removal (cellulose, phenol, phthalates, chlorinated organics, etc.). Naturally occurring microbial populations in soil, activated sludge and other samples are evaluated in the classification protocol and used as controls to establish baseline criteria for characterizing the bacterial additives.

The bioaugmentation products could either exhibit or not exhibit a significant difference from the controls. If there is no significant difference, then two possibilities are pursued:

- the protocol is not capable of discriminating between effective products and naturally occuring biota, or
- the products demonstrate no significant difference from naturally occuring biota.

Products with active biocultures are further evaluated with aerobic and/or parallel anaerobic activity tests, while non-bacterial products are evaluated with non-bacterial tests (nutrient analysis, tests for "free" enzyme activity). Appropriate short-term tests of enzyme activity are performed on homogenized cell-free extracts and filtrates (free

TABLE 10. COMPOSITION OF R2A MEDIUM

Ingredient	Concentration, g/L
Yeast Extract	0.5
Proteose Peptone No. 3	0.5
Casamino Acids	0.5
Glucose	0.5
Soluble Starch	0.5
Sodium Pyruvate	0.3
K_2HPO_4	0.3
$MgSO_4.7H_2O$	0.05
Agar	15.0

Final pH 7.2, adjust with K_2HPO_4 or KH_2PO_4 before adding agar. Add agar, heat medium to boiling to dissolve agar and autoclave for 15 minutes at 121°C, 15 psi.

enzymes) using procedures for determining enzyme activity contained in Methods of Enzymology as referenced in a manual published by Worthington Biochemical Corporation, Freehold, NJ 07728. A filter/ultrafiltration approach is used to isolate free enzymes from whole cells and cell fragments. Short-term tests of enzyme activity are completed on the filtrate. Longer term activity measurements on specific substrates are compared to "equivalent activated sludge" using whole cell tests based on disappearance of substrate and oxygen uptake determinations. The overall proposed approach for characterization and verification of product quality is shown in Figure 17.

The majority of bioaugmentation products have labels with general information concerning ingredients and broad performance claims. On labels where numbers of bacteria or levels of enzyme activity are listed, the procedures used for quantification are not reported. In addition, due to the lack of specificity, there have been reports by users of products that the additives were nothing more than inert fillers or dried activated sludge.

The specific goal of the label verification protocol is to provide simple and reproducible methods for measuring general microbial and enzymatic activity. Label verification is conducted "label blind," as if no product ingredients were specified. Protocols are being used to screen products where labels provide variable information on content. The verification of specific ingredients is an important part of the study.

The statistical experimental design developed with an appropriate quality control effectively addresses the testing of potency, consistency and microbial identification/enzyme characterization of the biocatalyst additives. Reproducibility of the testing methods to determine the potency and consistency of the biocatalysts is established by the use of ten replicates of standards, natural samples and products. The replicate samples are run initially to determine the variance of subsampling and analytical methods. Routine analyses during product testing are run in duplicate. The range of duplicate samples are compared to the standard deviation of the ten replicates. Results with a range exceeding two times the standard deviation are reported and the product sample is reanalyzed.

Product consistency is measured by analyzing ten replicate subsamples of ten different lots of the product requested from industry for the repeated testing. Suitable quality control in the Label Verification Protocol is established for the test methodologies used for plate counts, enumeration and identification of the active microbiota, enzyme activity, and whole cell tests.

The specifics of the Label Verification experimental design are presented in Figure 18. The design calls for three test sequences for all products; plate counts, enzyme activity, and whole cell activity tests.

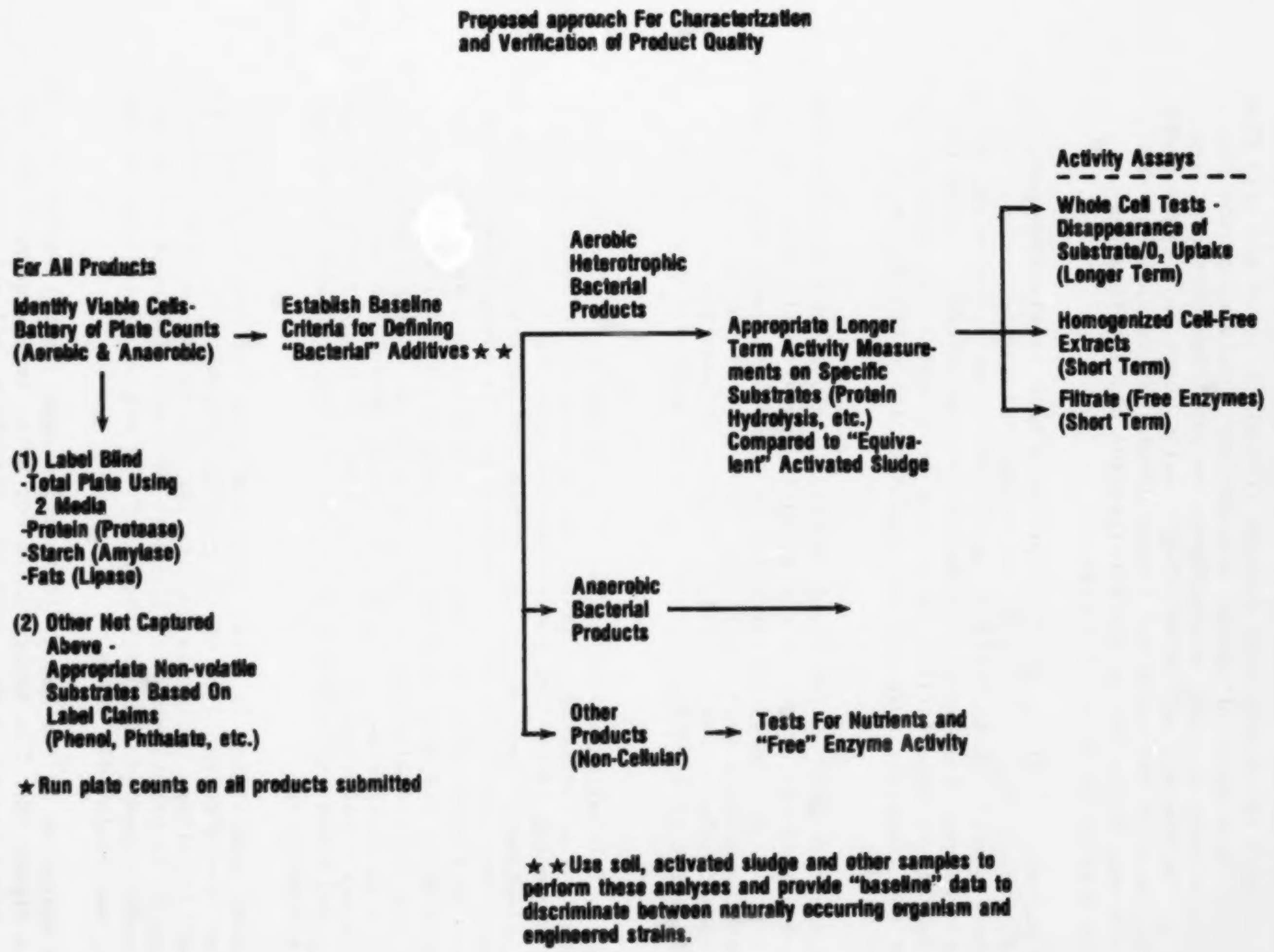
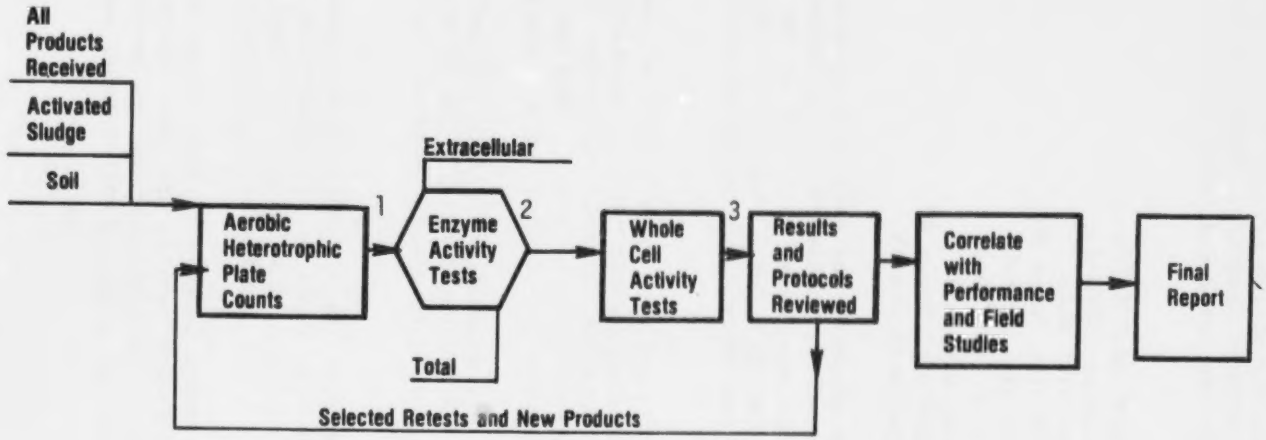

Proposed approach For Characterization and Verification of Product Quality
For All Products
Identify Viable Cells-Battery of Plate Counts (Aerobic & Anaerobic)
(1) Label Blind
-Total Plate Using 2 Media
-Protein (Protease)
-Starch (Amylase)
-Fats (Lipase)
(2) Other Not Captured Above - Appropriate Non-volatile Substrates Based On Label Claims (Phenol, Phthalate, etc.)
★ Run plate counts on all products submitted
Establish Baseline Criteria for Defining "Bacterial" Additives ★ ★
Aerobic Heterotrophic Bacterial Products
Anaerobic Bacterial Products
Other Products (Non-Cellular)
Appropriate Longer Term Activity Measurements on Specific Substrates (Protein Hydrolysis, etc.) Compared to "Equivalent" Activated Sludge
Tests For Nutrients and "Free" Enzyme Activity
Activity Assays
Whole Cell Tests - Disappearance of Substrate/O_2 Uptake (Longer Term)
Homogenized Cell-Free Extracts (Short Term)
Filtrate (Free Enzymes) (Short Term)
★ ★ Use soil, activated sludge and other samples to perform these analyses and provide "baseline" data to discriminate between naturally occurring organism and engineered strains.

Figure 17. Proposed Approach For Characterization and Verification of Product Quality.


All Products Received
Activated Sludge
Soil
Aerobic Heterotrophic Plate Counts
1
Enzyme Activity Tests
2
Extracellular
Total
Whole Cell Activity Tests
3
Results and Protocols Reviewed
Correlate with Performance and Field Studies
Final Report
Selected Retests and New Products

1.
Enumeration of organisms grown on general heterotrophic media and media which will enhance growth of organisms with selective enzymatic activity.

2.
Products are homogenized for total activity.
Products are ultrafiltered, filtrate is analyzed for extracellular activity.

3.
Batch activity tests include O_2 uptake rate, specific substrate disappearance, etc.

Figure 18. Label Verification Experimental Design.

Plate Counts

Protocol - Each product is analyzed initially with a series of plate counts. Samples are prepared by completely mixing 1 mL of liquid product or 1 gm of dry product to 99 mL of autoclaved distilled water. Dilutions are made as required. The plate counts are completed using nine different media:

Standard Plate Count Agar (STP)
STP Agar minus glucose (STPG-)
STPG- supplemented with milk
STPG- supplemented with Tween 80
STPG- supplemented with starch
Dilute strength STPG- (DSTPG-)
DSTPG- supplemented with milk
DSTPG- supplemented with Tween 80
DSTPG- supplemented with starch

Quality Control - Duplicate plates are prepared for each type of agar and each incubation time/temperature. Plates are incubated at two temperatures and two incubation periods. Duplicate plates with each of the media are incubated at 23°C for 48 hours, 23°C for 72 hours, 35°C for 48 hours and 35°C for 72 hours. Results are reported as organisms per unit weight or volume.

A positive and negative control is used with each analytical run. Positive controls are plates of agar and supplement with appropriate organisms (e.g. E. coli, Pseudomonas). Negative controls constitute plates of agar and supplement with no bacteria or products added. Routine quality control also consists of two subsamples (replicates) of a product.

Ten replicates of a liquid and a dry product are plated for each of the nine media. The ten repetitions are incubated at 25°C for 48 hours and 35°C for 48 hours. The results are used to develop control charts for 23°C and 35°C incubations.

The control charts are used to measure the variability of subsamples taken from a product. If the relative range of two subsamples exceeds three coefficients of variation for like samples (e.g. liquid samples incubated for 48 hours at 23°C), the samples are reanalyzed.

Ten replicates of five natural samples (e.g. activated sludge and soils) are analyzed. Three activated sludge samples are used (Northfield Township, Chelsea, and Ann Arbor). Two soil samples are analyzed. The results for these natural biomasses are compared with product sample results.

Enzyme Activity

Protocol - Extracellular and total enzyme activity are measured. Liquid and dry samples are prepared in the same way as samples for plate counting. Extracellular activity is measured on samples which have been filtered through a 0.45 micron filter. Total enzyme activity is measured on samples which have been completely homogenized in a high speed blender and then passed through a 0.45 micron filter.

Enzyme activity tests vary based on the enzyme of interest. However, most rely on measurement of the disappearance of a substrate or appearance of an enzymatic product. Results are reported in a number of ways, but generally relate to moles of substrate used or product produced per unit time. Standard enzymes are available for comparison.

Quality control - Routine analyses are completed in duplicate. A blank, three standards, and a matrix and control spike are included with each analytical run. Samples which exceed two times the activity of the highest standard are appropriately diluted and reanalyzed. Samples with activity less than 40 percent of the lowest standard are rerun either as a concentrated sample or with a diluted standard.

Ten replicates of the standard at three concentrations are analyzed to establish the reproducibility of the method. Ten replicates of a liquid and dry sample are analyzed to establish the reproducibility of subsampling procedures.

Whole Cell Tests

Protocol - The protocol depends on the product and its claims. Products making claims for specific substrate reductions are analyzed by the static-culture flask screening procedure (93, 94). Products with claims of more general performance are analyzed for oxygen uptake rate or TOC reduction. For either of these tests, products are added to real wastewater at the prescribed dose and at one-half and two times the prescribed dose. Three different wastewaters are used.

Quality Control - The reproducibility of the method is established with 10 replicates. Standard NSF laboratory analytical and quality control procedures are used when appropriate (e.g. BOD, TOC).

PERFORMANCE (EFFICACY) PROTOCOL

The specific tests in performance evaluation of bioaugmentation products in the municipal and industrial wastewater treatment include tests for enhancing: overall aerobic and anaerobic wastewater treatment; biodegradation of specific substrates; nitrification/denitrification; sludge reduction and settling; and, oxygen update by active microbiota in waste treatment.

Consequently, experimental design for performance testing protocol has two levels: to develop methodology for evaluating bioaugmentation product performance and to determine whether bioaugmentation products do improve performance of bench-scale extended aeration reactors and batch treatment systems.

The Performance (Efficacy) Protocol consists of: continuous flow studies for products claiming sludge reduction and better overall biological treatment; and batch biodegradability studies for products claiming improved treatment of specific organic priority pollutants and other hazardous and/or toxic compounds.

Continuous Flow Studies

The continuous flow studies incorporate in 20-liter capacity aeration reactors with separated clarifiers, synthetic and raw wastewater as media, specific pollutant substrates in several concentrations, and the bioaugmentation products. These products include specific substrate oxidizing products, sludge reduction products, sludge settling aid products, nitrification enhancement products, and O_2 uptake enhancement products. The tests at several product concentrations include several incubation temperatures as well as control systems without additives using the same specific substrates and activated sludge biomass in the synthetic and raw wastewater media.

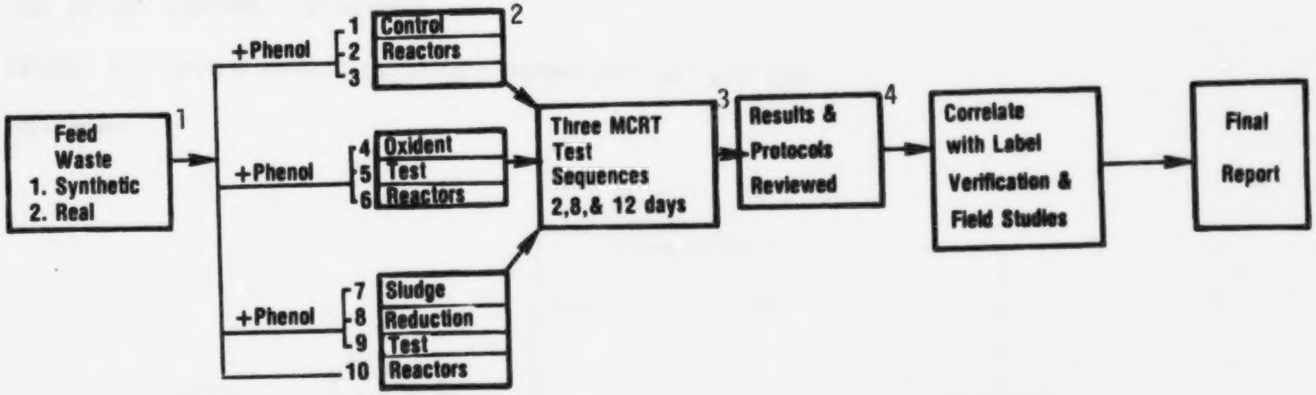
The performance protocol experimental design is outlined in Figure 19. The experimental design features: studies at 3 consecutive MCRT series (4, 8, and 12 days) (steady state achieved after 3 MCRT periods) to develop kinetics of specific substrate removal (substrate MCRT biodebiodegration rates) and kinetics of sludge reduction (sludge reduction rates); and studies to determine the effects of shock loads of substrates after completion of full test sequence with specific substrate.

The laboratory analyses include measurement of TOC/COD, BOD_5, SS/VSS of the effluent; D.O., pH, MLSS/MLVSS, ATP, dry solids, filterability and settleability of reactor liquor; as well as microscopic examiniation of the biomass of reactor liquor and waste sludge to characterize and identify the microbial population. Specific substrate analyses are performed on feed media, reactor liquor and effluent samples. Figure 20 describes the continuous flow testing phase of performance protocol sampling scheme and the reactor requirements.

The continuous flow studies with the synthetic and raw wastewater and twenty liter capacity aerated reactors with appropriate feed tanks, return sludge systems, clarifiers and collection tanks incorporate control systems with and without substrate, but without a bioaugmentation product; experimental reactors either for sludge reduction or sludge settling biocatalysts, nitrification/denitrification enhancement products, or oxygen uptake enhancement additives; but all with a specific substrate oxidizing product in them.

Experimental Design —

Steady state evaluation made at each of 3 consecutive MCRT series (steady state achieved after 3 MCRT periods), with measurements of effluent TOC; effluent specific substrate in the synthetic wastewater feed; effluent BOD_5; effluent SS and VSS; reactor biomass (MLVSS and ATP and microscopic


Feed
Waste
1. Synthetic
2. Real
1
+Phenol
+Phenol
+Phenol
1
2
3
Control
Reactors
2
4
5
6
Oxident
Test
Reactors
7
8
9
10
Sludge
Reduction
Test
Reactors
Three MCRT
Test
Sequences
2,8,& 12 days
3
Results &
Protocols
Reviewed
4
Correlate
with Label
Verification &
Field Studies
Final
Report

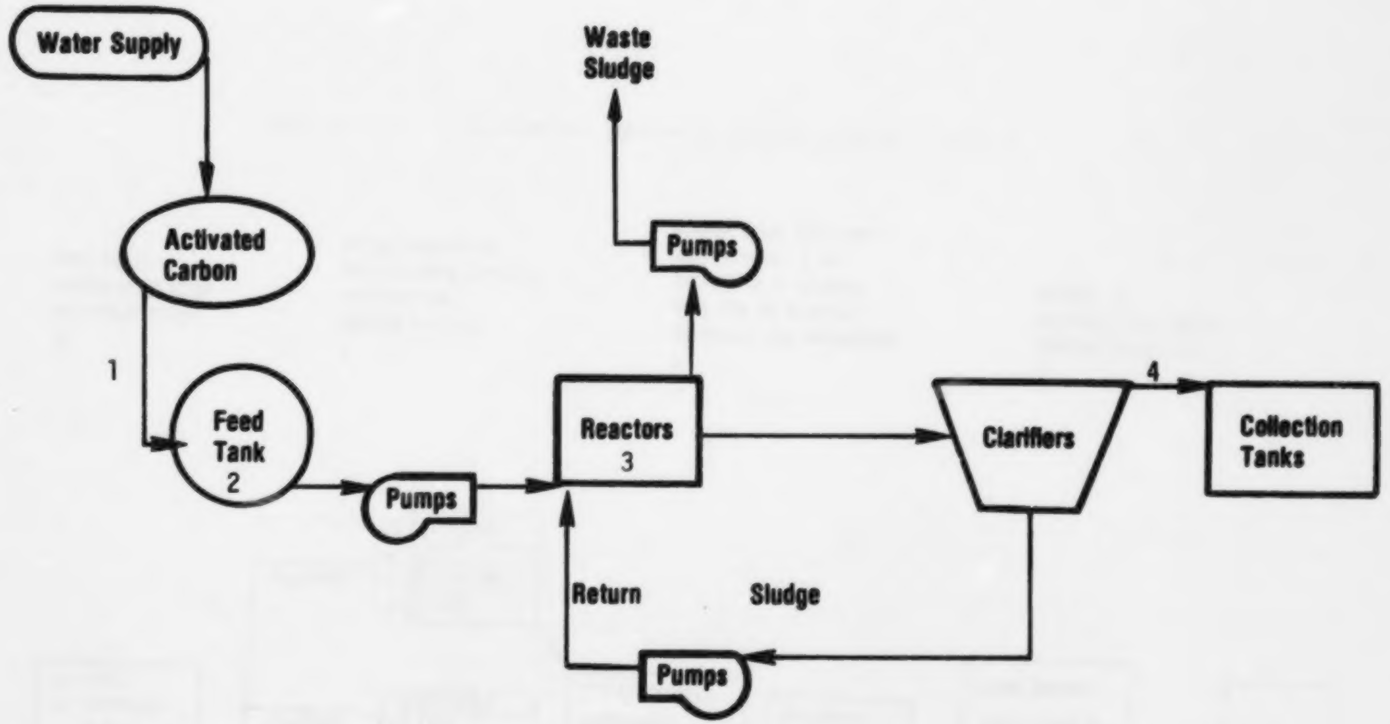
1.
Synthetic waste tested first, then real waste.

2.
Control and test reactors are interchanged between MCRT sequences.

3.
All MCRT test sequences aare run for 6 MCRT periods; 3 to achieve steady state, 3 for steady state operation.

4.
Results reviewed between each MCRT period.

Figure 19. Performance Protocol Experimental Design.


Water Supply
Activated Carbon
1
Feed Tank
2
Pumps
Reactors
3
Pumps
Waste Sludge
Clarifiers
4
Collection Tanks
Return
Sludge
Pumps

1. Chloramines

2. SS/VSS, BOD_5 (total & soluble), TOC (total & soluble), NH_4, NO_3, TKN, COD

3. ATP, SS/VSS, Filterability, Settleability

4. BOD_5 (total & soluble), TOC (total & soluble), NH_4, NO_3, TKN, COD, SS/VSS

Figure 20. Performance Protocol Sampling Scheme.

characterization of organisms); waste sludge dry mass; waste sludge filterability; sludge settling characteristics using settleometer (ZSV).

- Shock load, after completion of full test sequence (period of 6 MCRT) with specific substrate.

The elements of the testing method using 10 reactors include:

- Consecutive MCRT series of 4 days, 8 days and 12 days with reactor hydraulic retention times of 6 hours;
- Steady state established after 3 MCRT periods, with 3 samples analyzed per each MCRT period before steady state is achieved;
- After steady state is attained, 9 samples collected during the next 3 MCRT periods;
- Five gallon (20 liter) aeration reactors with an external clarifier, and pumps to return settled sludge to the reactor;
- Sludge settling tests with the withdrawn sludges that are returned to the reactor;
- Sludge wasting from the reactor, with the volume and frequency based on the MCRT and pump characteristics;
- Synthetic wastewater for the continuous flow studies as formulated and used by U.S. EPA, Cincinnati, Ohio (95, 96).

The composition of the synthetic wastewater is described in Table 11. A detailed sampling schedule and description of analysis to be performed on wastewater feed, reactor liquor and effluent samples is shown in Table 12.

A statistical experimental analysis design for the continuous feed portion of the efficacy testing protocol, based on Latin Square approach, comprises studies with synthetic and raw wastewatwer systems at 3 MCRT test sequences (4, 8 and 12 days), each to be run for six MCRT periods; 3 to achieve steady state and 3 during steady state operation. The study variables in the experimental statistical design are as follows: type of media: synthetic wastewater (S); raw wastewater (W); treatment methods: control (C); sludge reduction (R); substrate oxidation (O); sludge settling action (A); aerobic treatment (AT); anaerobic treatment (ANT); nitrification/denitrification activity (N); oxygen uptake activity (U); and 3 different MCRT series to be run sequentially (4-, 8- and 12-day series.

Reactors with synthetic and raw wastewater media are run either sequentially or concurrently. Because of the different wastewater conditions, the results for the synthetic and raw wastewater tests are

TABLE 11. SYNTHETIC WASTEWATER FORMULATION

Used in the Continuous Flow Biodegradation Studies at U.S. EPA,
WERL, Wastewater Research Division Laboratories

In order to eliminate the variable nature of municipal wastewater and prevent possible introduction of toxic materials during the acclimation period, a synthetic waste was used in most of these studies. This feed solution was formulated after a review of prior published formulations (10 through 23).

The final synthetic waste solution, prepared by diluting stock solutions with distilled water had the following composition:

Constituent	Concentration, mg/L
KH_2PO_4	8.5
K_2HPO_4	22
Na_2HPO_4	33
NH_4Cl	2
$MgSC_4$	22
$CaCl_2$	36
$FeCl_3$	0.3
Urea	50
$NaHCO_3$	300
Yeast Extract (Difco)	55
Baco Peptone (Difco)	50
Meat Extract (Difco)	50
Fish Meal Extract (Purina Trout Chow)	0.5 ml

The fish meal extract was prepared by grinding 10g of Purina Trout Chow with 200 ml of distilled water in a high speed blender for 3 min. The mixture was allowed to settle for 10 min. and then 0.5 ml added to each liter of synthetic waste. Experience has shown this additive helps control bulking of sludges produced from synthetic feeds.

The synthetic feed typically has the following characteristics:

Item	Concentration, mg/L
Chemical oxygen demand	160
Total organic carbon	76
Suspended solids	2
Total Kjeldahl nitrogen	47
Ammonia nitrogen	2
Nitrite and nitrate nitrogen	<0.1
Total phosphorus	13
Alkalinity, as calcium carbonate	211
pH	7.3 units

The nitrogen and phosphorus content is slightly higher than most municipal wastewaters. However, it was deemed necessary to have these nutrients in excess to insure the waste was not growth limiting. The suspended solids are low because the waste is composed of soluble materials for ease of preparation of the feed from stock solutions.

TABLE 12. PROPOSED LAB ANALYSES

ANALYSIS	SAMPLE LOCATION	SAMPLE TYPE	SAMPLE FREQUENCY	WASTEWATER FEED
TOC	Clar. Eff.	Grab	(1), (2)	(3), (4)
BOD_5	Clar. Eff.	Grab	(1), (2)	(3), (4)
SS/VSS	Clar. Eff.	Grab	(1), (2)	(4)
D.O.	Reactor	Probe	Daily/Reactor	-
pH	Reactor	Probe	Daily/Reactor	-
MLSS/MLVSS	Waste Sludge	Grab	Daily/Reactor	-
ATP	Waste Sludge	Grab	(1), (2)	-
Dry Solids	Waste Sludge	Grab	(1)	-
Filterability	Waste Sludge Or Reactor	Grab*	(1)	-
Settleometer (Sludge Settleability)	Reacter	Grab*	(1), (2)	-
Slide for Microscopic Examination	Waste Sludge	Grab	(1)**, (2)	

Key to Sample Frequency/Wastewater Feed:

(1) Analyses to be completed 18 times over a period of 3 MCRT's to achieve a steady state and 3 MCRT's at a steady state.
(2) Analyses to be completed during transition periods at a frequency of 3 times/MCRT.
(3) Periodic check for synthetic waste - 1 time/month or less if uniform results obtained.
(4) Perform tests as needed to characterize wastewater feed. Use WWTP data as a QC.

* Sample returned to reactor upon completion of test.

** Prepare 1 slide for each MCRT at steady state (total of 3).

analyzed as two separate experiments. In order to increase the statistical power of the experimental design, the results of the separate analyses are combined, where possible, to yield an overall estimate of efficacy for a specific treatment and an overall test statistic comparing the experimental systems to control systems.

In the Latin Square design, ten reactors allow for testing of two different biodegradation products at the same time per experiment. Three reactors are operated for each bioaugmentation product and three as controls. The design includes 9 experimental reactors with a tenth reactor as an additional control for the sludge reduction product.

Currently there are two bioaugmentation products tested for performance in comparison to the control systems, a sludge reducing product and a specific substrate oxidizing product. Accordingly, an example of the experimental and control reactor strategy for the specific oxidant (i.e. phenol) and sludge reduction product in one experiment is illustrated as follows:

1	2	3	4	5	6	7	8	9	10
O	C_1	R	O	C_1	R	O	C_1	R	C_2

C_1 = control with substrate
C_2 = control without substrate

O = specific substrate oxidant
R = sludge reduction additive

The tenth reactor, used as a control in the monitoring of the impact of a specific substrate on a sludge reduction product, is operated with a wastewater that does not have an added substrate. The control reactors 2, 5 and 8 receive wastewater spiked with a specific substrate.

The sampling schedule in the proposed performance testing protocol consists of 18 samples during each MCRT series (4, 8 and 12 days). An MCRT series consists of 3 MCRT periods to approximate steady state followed by 3 MCRT periods for product evaluation. The analysis series of 18 samples for each series provides kinetic data for a treatment system with and without the use of a specific biocatalytic additive. The general Latin Square design (as shown in Figure 21) provides adequately an interchange between control and test reactors at the end of each MCRT series and generates statistically sound experimental data. The specific oxidant used in the first series of experiments is phenol oxidizing products with a phenol substrate at 50 mg/L.

The MCRT is used as a blocking factor. The design calls for three distinct MCRT series of 4, 8 and 12 days in succession for each reactor. This gives a range of MCRT within a reasonable period of time for the overall experiment. The treatment used in a reactor is changed when the MCRT changes.

LATIN SQUARE EXPERIMENTAL DESIGN
FOR THE PERFORMANCE PROTOCOL.

MCRT (days)	Reactor ID								
	1	2	3	4	5	6	7	8	9
4	S	C	R	S	C	R	S	C	R
8	C	R	S	C	R	S	C	R	S
12	R	S	C	R	S	C	R	S	C

C = control

R = sludge reduction product

S = specific substrate oxidizing product

Figure 21. Latin Square Experimental Design for the Performance Protocol

The study design is a repeated measures design with successive measurements taken for each reactor under differing conditions. Each reactor is run at the three MCRT in succession during an experiment. For a given waste type, the experimental conditions are the same for all reactors in operation at a given time, except the treatments will be different for different reactors. Although the conditions of operation for the various reactors are controlled to be as similar as possible, except for the treatment, it is appropriate to consider the reactor to be a blocking factor.

Quality Control -- Routine performance testing quality control includes analytical quality control and reactor operation quality control. Analytical quality control procedures are specified in the NSF Analytical Services Quality Control Manual and the NSF Chemistry Laboratory Methods Manual. Reactor operation quality control procedures are specified in the NSF quality control document.

Batch Biodegradability Studies

The Batch Biodegradability Studies are performed to measure possible enhancement of the specific substrate disappearance under aerobic and anaerobic conditions through the use of substrate oxidizing or reducing bioaugmentation products. In aerobic batch biodegradation testing, the electrolytic respirometry approach in which oxygen uptake by the microbiota is used for indirect determination of percent biodegradation, and the batch-shaker flask method are employed. The batch-shaker flask method testing uses specific substrate analyses or indirect methods such as TOC and/or DOC determination of culture samples.

With the electrolytic respirometry method, generated oxygen uptake velocity data are used to calculate BOD values for respective incubation times, at several incubation temperatures of culture media containing the organic test compound. The percent biodegradation of the substrate is determined for same-time intervals from the BOD and ThOD (theoretical oxygen demand) ratio. In addition, oxygen uptake data as well as the concentration of active biomass are used to develop BOD kinetic coefficients for determination of specific growth rates and biodegradation rates of the substrate with or without the bioaugmentation products.

The electrolytic respirometry biodegradation testing experimental design is outlined in Figure 22. Control biomass used in the studies is: activated sludge; soil inoculum; or enrichment culture. The studies incorporate the use of controls with substrate without biomass, or controls without substrate with either biomass and product together or with biomass alone. The experimental reactor systems with the substrate oxidizing product use substrate, biomass and the product at several concentration levels. The experimental reactor systems without the product will incorporate reactors containing substrates and biomass at the same concentration levels for parallel monitoring of substrate disappearance in reactors with or without the bioaugmentation product. The biodegradation products are compared against: controls with substrate; controls without

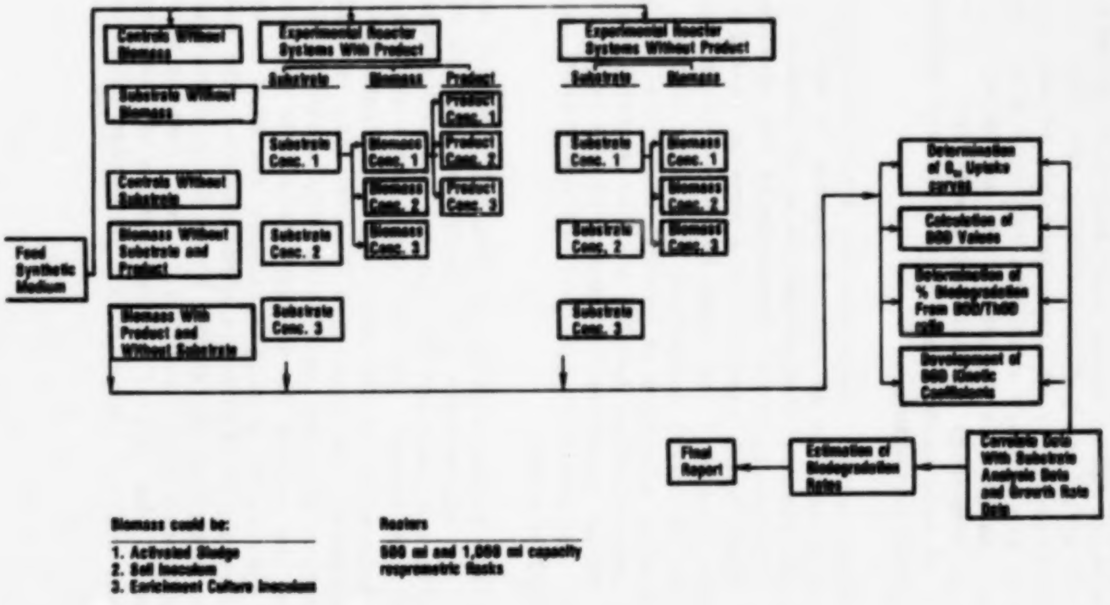

Feed Synthetic Medium
Controls Without Biomass
Substrate Without Biomass
Controls Without Substrate
Biomass Without Substrate and Product
Biomass With Product and Without Substrate
Experimental Reactor Systems With Product
Substrate
Biomass
Product
Substrate Conc. 1
Substrate Conc. 2
Substrate Conc. 3
Biomass Conc. 1
Biomass Conc. 2
Biomass Conc. 3
Product Conc. 1
Product Conc. 2
Product Conc. 3
Experimental Reactor Systems Without Product
Substrate
Biomass
Substrate Conc. 1
Substrate Conc. 2
Substrate Conc. 3
Biomass Conc. 1
Biomass Conc. 2
Biomass Conc. 3
Determination of O₂ Uptake curves
Calculation of BOD Values
Determination of % Biodegradation From BOD/ThOD ratio
Development of BOD Kinetic Coefficients
Correlate Data With Substrate Analysis Data and Growth Rate Data
Estimation of Biodegradation Rates
Final Report
Biomass could be:
1. Activated Sludge
2. Soil Inoculum
3. Enrichment Culture Inoculum
Reactors
500 ml and 1,000 ml capacity respirometric flasks

Figure 22. Batch Electrolytic Respirometry Biodegradability Experimental Design.

substrate; controls without product; activated sludge and/or soil inoculum of "equivalent dose"; and, enrichment cultures as inoculum of "equivalent dose."

Synthetic medium (Table 13) used in the studies was formulated to meet Organization for Economic Cooperation and Development (OECD) standards and consists of mineral salts, trace salt solution, vitamin solution and/or yeast extract. In studies utilizing the cometabolism approach to discern biodegradability of the substrate, vitamin and/or yeast substrate serves as the primary substrate for growth and energy source of microorganism. As that is depleted, the microbiota turn to secondary substrate (test compound) for metabolic activity. In studies using the test compound as sole carbon source, the primary substrates are deleted from the synthetic medium.

The batch-shaker flask method, incorporating activated sludge or soil or enrichment culture inoculum as control biomass in synthetic medium, is an approach for testing biodegradation as developed by OECD and modified at USEPA, Cincinnati, Ohio. Essentially the experimental approach of the shaker flask batch system is the same as that for the electrolytic respirometry studies. The flask batch method employs Erlemeyer flasks (2-liter capacity) containing one liter volumes of synthetic medium spiked with substrate at several concentration levels, and biomass at several concentration levels, with or without the bioaugmentation product. Three concentration levels of the product are considered in the test. Activated sludge, soil or enrichment culture inoculum serve as control biomass.

The batch-shake flask biodegradation testing experimental design is outlined in Figure 23. At appropriate time intervals, samples of thoroughly mixed liquid media are analyzed for residual specific substrate and residual TOC/DOC in the system. For the same time intervals, levels of active biomass are determined in the same samples to estimate growth rate biokinetic coefficients. The generated TOC/DOC data as well as specific residual substrate data are used to develop substrate loss curves and to determine the percent by degradation of the substrate. The generated data on the levels of active biomass are used to provide growth rate curves and estimate biokinetic coefficients. The substrate removal and growth rate data for the Shaker Flask Batch System are then corelated with data generated from electrolytic respirometry studies to make a final assessment of the rate of biodegradation of the substrate in the reactors with and without the substrate oxidation enhancement bioagumentation product.

FIELD TESTS

The NSF will not conduct field evaluations of bioaugmentation products within the scope of this project. However, independent data from treatment facilities already using bioaugmentation products will be obtained and reviewed by NSF.

TABLE 13. COMPOSITION OF SYNTHETIC MEDIUM
Manometric Respirometry (Rev 5)

1.6.1.2. Nutrient solution.

The nutrient solution contains per litre, 10 ml of solution (a) and 1 ml of each of the following solutions (b) to (f) in water (1.6.1.1.) (A.R. means Analytical reagent)

(a)	KH_2PO_4	AR	8.50 g
	K_2HPO_4	AR	21.75 g
	$Na_2HPO_4.2H_2O$	AR	33.40 g
	NH_4Cl	AR	2.50 g
	dissolve in and made up to 1000 ml with water (1.6.1.1.) The pH value should be pH: 7.2		
(b)	$MgSO_4.7H_2O$	AR	22.50 g
	dissolve in and make up to 1000 ml with water (1.6.1.1.)		
(c)	$CaCl_2$	AR	27.50 g
	dissolve in and made up to 1000 ml with water (2.6.1.1.)		
(d)	$FeCl_3.6P._2O$	AR	0.25 g
	dissolve in and make up to 1000 ml with water (1.6.1.1.) This solution is freshly prepared immediately before use.		

(continued)

TABLE 13. COMPOSITION OF SYNTHETIC MEDIUM
Manometric Respirometry (Rev 5) (cont'd)

(e) Trace element solution

$MnSO_4.4H_2O$	AR	39.9 mg (=30.23mg $MnSO_4$)
H_3BO_3	AR	57.2 mg
$ZnSO_4.7H_2O$	AR	42.8 mg
$(NH_4)_6MO_7O_{24}$ (=36.85mg $(NH_4)_6MO_7O_{24}.4H_2$	AR	34.7 mg
Fe - chelate:		
($FeCl_3$.EDTA)	AR	100 mg

dissolve in and make up to

1000 ml with water (1.6.1.1.)

Sterilization of the trace element stock solution at (120°C),

2 atm. 20 min.

(f) Vitamin solution

Biotin	AR	0.2 mg
Nicotinic acid	AR	2.0 mg
Thiamine	AR	1.0 mg
p-Aminobenzoic acid	AR	1.0 mg
Pantothenic acid	AR	1.0 mg
Pyridoxamine	AR	5.0 mg
Cyanocobalamine	AR	2.0 mg
Folic acid	AR	5.0 mg

dissolve in and make up to 100 ml with water, (1.6.1.1.)

The solution is filtered sterile through 0.2 m membrane filters

Instead of solution 1.6.1.2. (f) 15 mg of yeast extract may be used per 100 ml of water (1.6.1.1.)

Solutions (e) and (f) may be omitted.

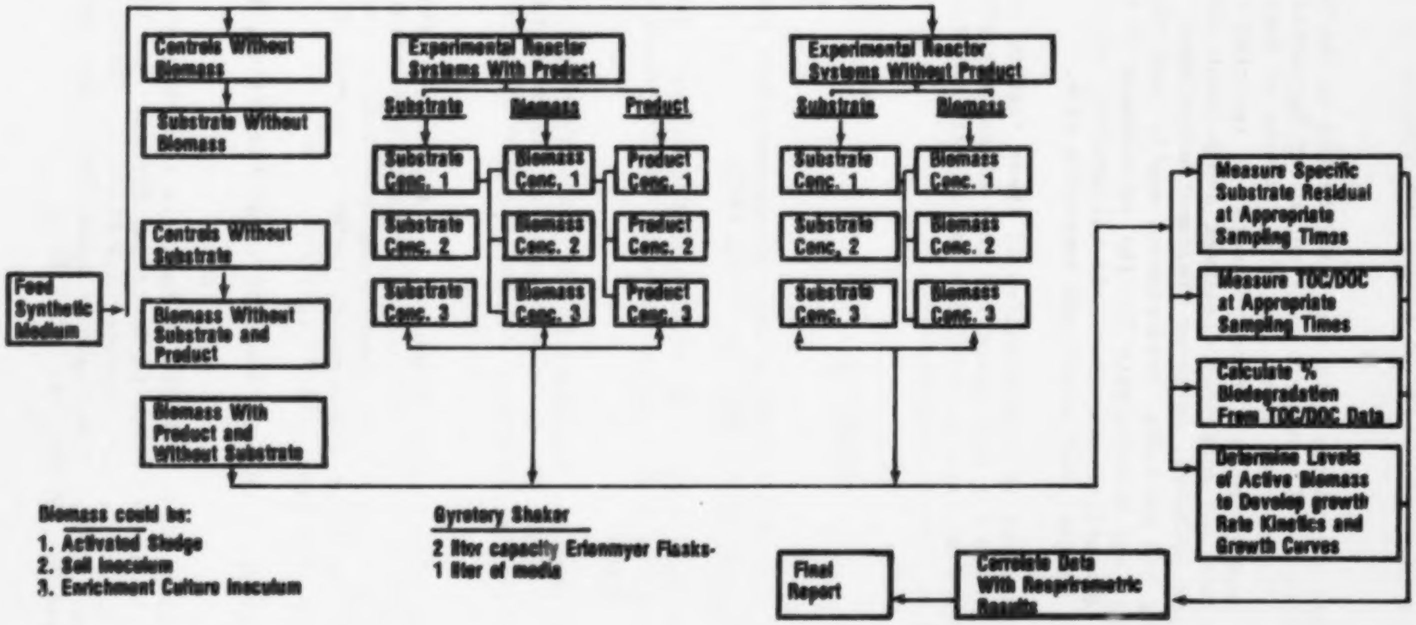

Feed Synthetic Medium
Controls Without Biomass
Substrate Without Biomass
Controls Without Substrate
Biomass Without Substrate and Product
Biomass With Product and Without Substrate
Experimental Reactor Systems With Product
Substrate
Biomass
Product
Substrate Conc. 1
Substrate Conc. 2
Substrate Conc. 3
Biomass Conc. 1
Biomass Conc. 2
Biomass Conc. 3
Product Conc. 1
Product Conc. 2
Product Conc. 3
Experimental Reactor Systems Without Product
Substrate
Biomass
Substrate Conc. 1
Substrate Conc. 2
Substrate Conc. 3
Biomass Conc. 1
Biomass Conc. 2
Biomass Conc. 3
Measure Specific Substrate Residual at Appropriate Sampling Times
Measure TOC/DOC at Appropriate Sampling Times
Calculate % Biodegradation From TOC/DOC Data
Determine Levels of Active Biomass to Develop growth Rate Kinetics and Growth Curves
Correlate Data With Respirometric Results
Final Report
Biomass could be:
1. Activated Sludge
2. Soil Inoculum
3. Enrichment Culture Inoculum
Gyratory Shaker
2 liter capacity Erlenmyer Flasks-
1 liter of media

Figure 23. Batch Shaker Flask Biodegradability Experimental Design.

CURRENT STATUS IN BIOAUGMENTATION EVALUATION STUDIES

Currently 12 bioaugmentation products are being tested by the Label Verification testing protocol. The biocatalysts represent bacterial cultures alone, bacterial cultures with nutrients, and mixtures of bacterial cultures and enzymes. These bioaugmentation products, as reported by the industries, serve as aerobic and anaerobic treatment enhancement additives, sludge handling aid products, in-sewer treatment enhancement products, sludge settling aid products, deodorizers, septic tank treatment enhancement products, and biocatalysts for the enhancement of the treatment of phenolics, aromatics, petrochemicals (aliphatic), pulp and paper mill wastes, dairy wastes, and animal and vegetable oils.

The successful development and confirmation of these testing protocols will provide an approach for evaluating not only bioaugmentation products currently produced by natural selection and in situ mutation, but also those produced by genetic engineering.

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MUNICIPAL SLUDGE OXIDATION WITH THE VERTICAL TUBE REACTOR

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ABSTRACT

The first full-scale vertical tube reactor (VTR) was built at Longmont, Colorado in 1983 for wet oxidation of municipal wastewater treatment sludge. The VTR consists of coaxial pipes about 2400 meters long suspended vertically in a deep well.

The concentric annular spaces form very long U-tubes. Sludge and air are pumped down through an annular space and return up through an adjacent annular space.

When preheated to a sufficiently high temperature, wet oxidation occurs, destroying up to 80% of the chemical oxygen demand (COD) of the sludge. Readily settleable ash and short chain organic acids are formed. The clarified VTR effluent liquor is returned to the municipal wastewater treatment plant where it is treated through the existing biological secondary treatment units.

Temperatures above 260°C can be attained at the bottom of the VTR without boiling because of the high hydrostatic pressure developed by the column of liquid. Higher temperatures produce greater percentage reductions of the COD. The wet oxidation reaction is autogenic after the reactor is initially heated using an external heat source.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

INTRODUCTION

The City of Longmont, Colorado entered into cooperative agreements with the United States Environmental Protection Agency (USEPA) and a private industry (VerTech) in 1982. By these agreements, a prototype vertical tube reactor (VTR) for wet oxidation of the City's municipal wastewater treatment plant sludge was designed and constructed by VerTech's predecessor in 1983. After initial operation, VerTech redesigned and retrofitted the reactor in 1984. Demonstration of the VerTech System with USEPA grant assistance is scheduled to be completed by December 1985.

Prior to the construction of the prototype VTR, VerTech's predecessor had developed and operated a bench scale laboratory reactor and a small 2" pilot reactor which they constructed in an abandoned oil well. The results of this earlier work encouraged Bow Valley, VerTech's parent company to finance the construction of the prototype facility. Early operating results further encouraged Bow Valley to acquire the process patent rights and to form VerTech Treatment Systems to commercialize the process.

The City of Longmont, Colorado was chosen as the site for the prototype installation for a number of reasons. The City is located thirty miles north of Denver, a short travel distance for private industry. Due to rapid growth, the City was facing a growing sludge disposal problem. The City with a population of 46,000 has a representative combination of residential, commercial and industrial users of the City's municipally owned wastewater treatment system. The size of the reactor the industry wanted to build for the demonstration had the potential to become the primary means of treatment for all of the City's sludge. The City is within easy haul distance of a number of diverse industrial waste producers so that the VTR at that location may be used to demonstrate the process to handle a variety of wastes. As an additional important consideration, the City staff and elected officials were enthusiastic about being a part of a cooperative effort between the local community, VerTech, and the USEPA to develop and demonstrate this innovative process.

Under the cooperative agreements, the City provided the land at its wastewater treatment plant, plant modifications to accommodate the VTR, laboratory facilities and personnel, prepared the grant application, and administered the grant funds provided by USEPA.

VerTech designed and built the VTR at its own expense and furnishes the personnel to operate it.

The USEPA is furnishing grant funds to help offset the City's costs and VerTech's cost of operation during the demonstration period.

The firm of McCall-Ellingson and Morrill, Incorporated, is the City's consulting engineer in this project which has been designated as principal investigators to prepare the final report.

THE VERTICAL TUBE REACTOR

VerTech's below-ground system (Figure 1) is housed in a conventionally drilled, cement encased well. Tubes of various diameters are concentrically arranged within the well to create annular spaces for two-phase flow and heat exchange.

The influent, composed of diluted liquid sludge and air, is pumped into the inner annular space under low pressure. The influent stream is pressurized to 9.65 MPa (1400 psi) at the bottom of the well by the height and density of the fluid column above. Oxidation takes place when oxygen and organic constituents are present at a sufficient temperature. Temperatures above 260°C (500°F), can be attained without boiling because of the high hydrostatic pressure that is developed. The result is wet air oxidation with a dramatic reduction of organic solids to a small ash residue. Excess heat liberated during the process is transferred to the surface by an internal hot oil heat exchanger.

Tube diameters and overall lengths are designed to provide sufficient residence time to complete the oxidation process. The high length-to-diameter ratio of 30,000:1 provides efficient counterflow heat exchange, mass transfer, and plug flow to maximize chemical reaction.

A significant advantage of a long reaction vessel installed in a deep well is the influent sludge and air or oxygen can be introduced near the top of the reaction chamber at nominal pressure of 2.1-3.4 MPa (300-500 psi) with maximum pressures equal to 9.7-10.3 MPa (1400-1500 psi) developed at the bottom of the reaction chamber due to hydrostatic pressure of the column of waste.

THE CITY OF LONGMONT FACILITY

Figure 2 is a flow diagram of the Longmont installation. Sludge from the City plant is stored in a mechanically mixed holding tank from which it is pumped at a controlled rate to the vertical tube reactor.

Diluent in the form of City plant process water, City plant effluent or reactor effluent recycle is mixed with the sludge to produce the desired influent concentration to the reactor.

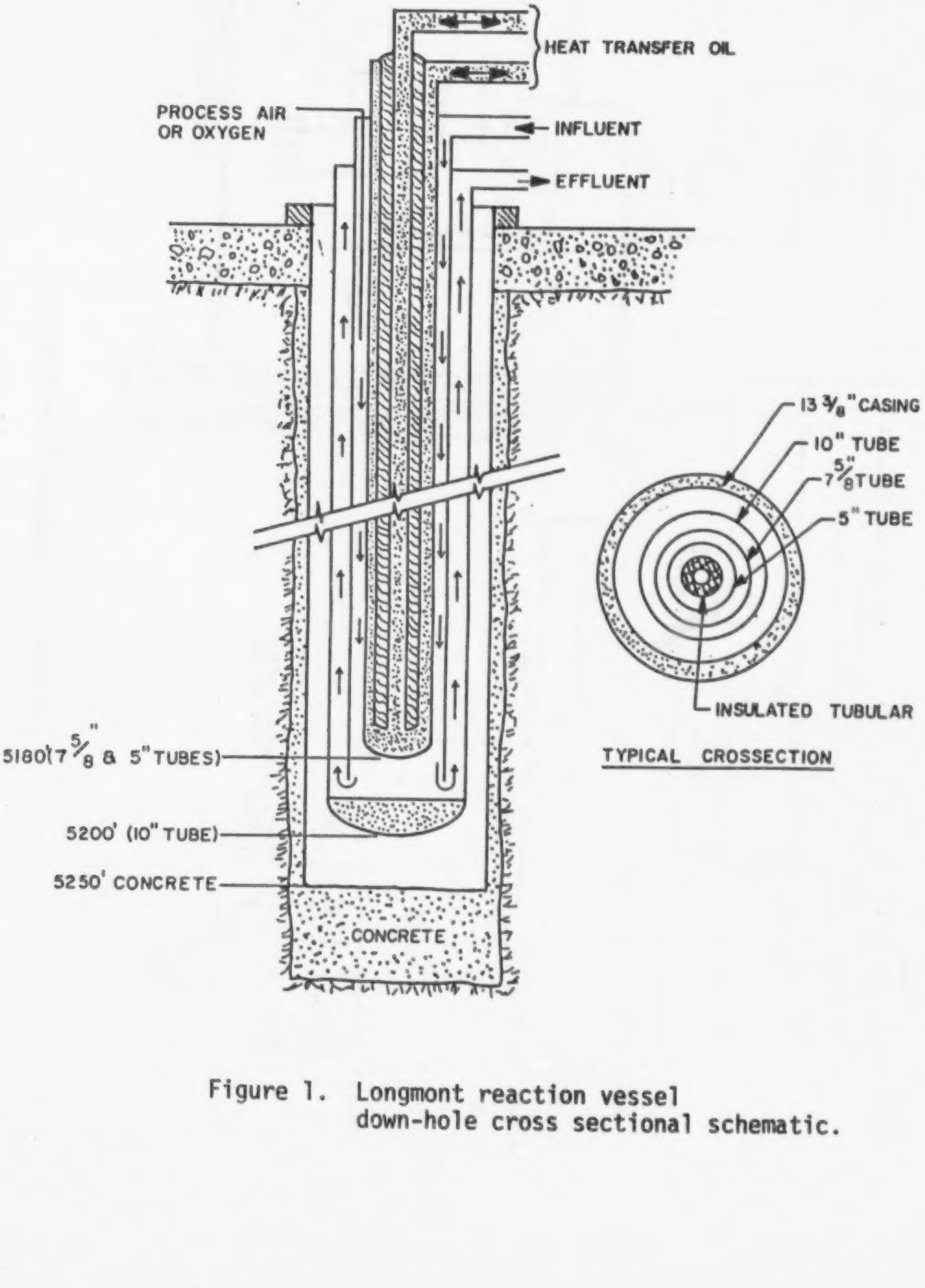
HEAT TRANSFER OIL
PROCESS AIR OR OXYGEN
INFLUENT
EFFLUENT
13 3/8" CASING
10" TUBE
7 5/8" TUBE
5" TUBE
INSULATED TUBULAR
TYPICAL CROSSECTION
5180'(7 5/8" & 5" TUBES)
5200' (10" TUBE)
5250' CONCRETE
CONCRETE

Figure 1. Longmont reaction vessel
down-hole cross sectional schematic.

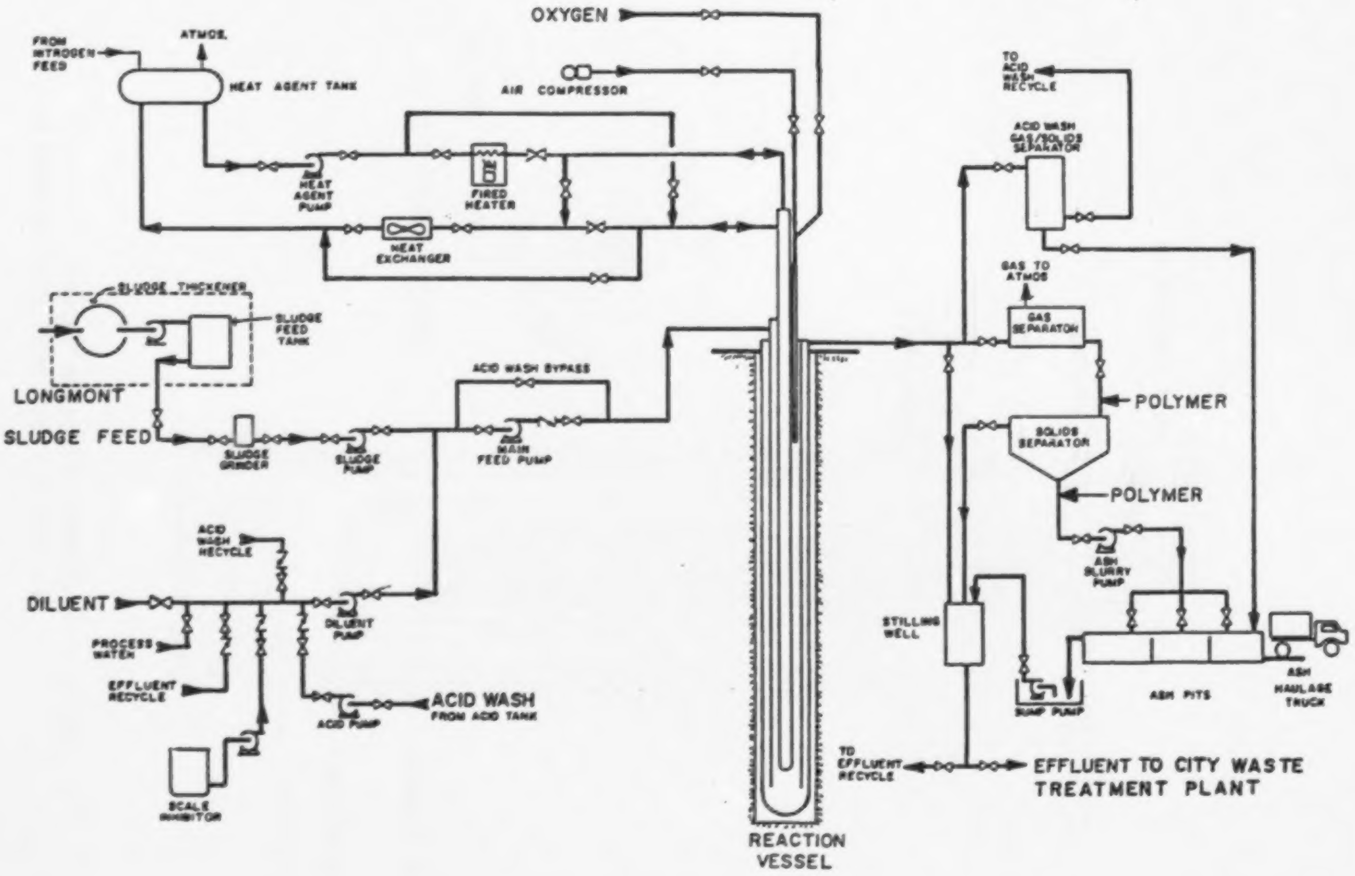
OXYGEN
FROM NITROGEN FEED
ATMOS.
HEAT AGENT TANK
AIR COMPRESSOR
HEAT AGENT PUMP
FIRED HEATER
HEAT EXCHANGER
SLUDGE THICKENER
SLUDGE FEED TANK
LONGMONT
SLUDGE FEED
SLUDGE GRINDER
SLUDGE PUMP
ACID WASH BYPASS
MAIN FEED PUMP
ACID WASH RECYCLE
DILUENT
DILUENT PUMP
PROCESS WATER
EFFLUENT RECYCLE
ACID WASH
FROM ACID TANK
ACID PUMP
SCALE INHIBITOR
REACTION VESSEL
TO ACID WASH RECYCLE
ACID WASH GAS/SOLIDS SEPARATOR
GAS TO ATMOS
GAS SEPARATOR
POLYMER
SOLIDS SEPARATOR
POLYMER
ASH SLURRY PUMP
STILLING WELL
SUMP PUMP
ASH PITS
ASH HAULAGE TRUCK
TO EFFLUENT RECYCLE
EFFLUENT TO CITY WASTE TREATMENT PLANT

Figure 2. Process flow diagram.

The reactor feed pump is a positive displacement variable speed pump which is used to control the flow rate through the reactor. The sludge pump is also a positive displacement variable speed pump by which the rate of sludge feed to the reactor is controlled. The diluent pump is a centrifugal constant speed pump. The diluent delivered to the system makes up the difference between the volume pumped by the sludge pump and the volume pumped by the reactor feed pump. The amount of chemical oxygen demand (COD) in the feed to the VTR is adjusted simply by changing the speed of the sludge pump.

Compressed air was initially used as the only source of oxygen. An oxygen source (Figure 3) has since been added so that oxygen-enriched air can be provided. The oxygen source is introduced at 30 or 122 meters (100 or 400 feet) from the surface. Temperature and pressure to start the oxidation reaction is developed at about 300 meters (1,000 feet) below the surface.

A heat exchanger is provided to add heat to or extract heat from the reactor. Heat transfer oil is circulated through the heat exchanger which extends essentially the full depth of the reactor (Figure 1). Heat can be added by a natural gas fired heater or wasted through a fan powered radiator to the atmosphere.

Energy recovery has been demonstrated at Longmont, but the excess heat provides no beneficial use.

For startup of the reactor it is necessary to add heat to bring the reactor up to operating temperature and to heat the rock surrounding the reactor. At Longmont it was necessary to add heat continuously through the early stages of the demonstration project. In fact, the system did not operate autogenically until after the oxygen-enrichment system was added.

Effluent leaves the reactor at a temperature of about 11-14C° above the influent temperature. Much of the reaction zone heat is given up to preheat the incoming waste. The VTR effluent is routed through a gas separator and a solids separator and is then returned to the City wastewater treatment plant.

Ash from the bottom of the separator is pumped as slurry to ash pits. Clarified liquor is decanted and returned to the City wastewater treatment plant. Settled ash is periodically removed and taken to the City landfill or other ultimate disposal. The ash is essentially inert. The combination of wet air oxidation to remove biological suspended solids, coupled with ash settling to 40 wt%, provides for a 25-fold reduction in solids volume.

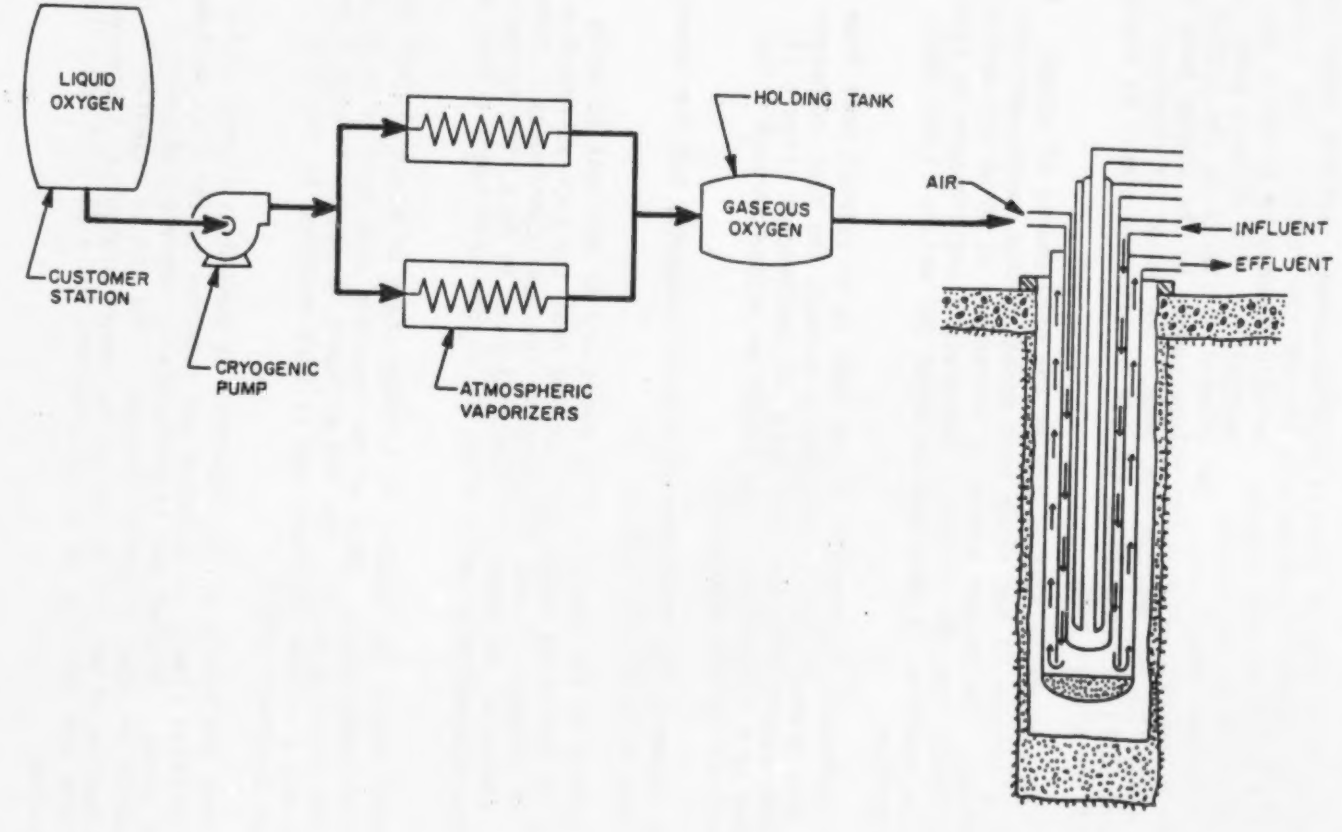
LIQUID OXYGEN
CUSTOMER STATION
CRYOGENIC PUMP
ATMOSPHERIC VAPORIZERS
HOLDING TANK
GASEOUS OXYGEN
AIR
INFLUENT
EFFLUENT

Figure 3. Oxygen-enriched air flow diagram.

The liquor returned to the City wastewater treatment plant contains low molecular weight organic acids (C_1 to C_4), primarily acetic acid. The strength of the liquor varies from 2,000 mg/l to 6,000 mg/l of COD, depending on the reactor feed concentration. The liquor strength varies directly with the VTR loading. The COD percentage reduction increases with inlet concentration and with higher reactor operating temperature, as shown on Figure 4. Higher operating temperatures also form shorter chain acids in the reactor effluent.

Provision is made to recycle clarified VTR effluent to partially or totally replace the other diluent sources. Recycling, initially practiced to conserve process heat, appears to reduce the organic acids to shorter chain acids and also to oxidize some of the ammonia formed in a single pass operation.

VerTech has operated bench scale completely mixed activated sludge bio-reactors at the Longmont facility from August, 1984 through May, 1985. This has confirmed the treatability of the clarifier reactor effluent. The reduction of biochemical oxygen demand (BOD) and COD through the VTR and bench scale bio-reactor, is shown by the schematic diagram on Figure 5. The overall BOD reduction from the diluted municipal sludge at 11,800 mg/l to the bench scale bio-reactor effluent of 76 mg/l is greater than 99%.

There is no reason to believe the treatment through the municipal plant is not comparable to that through the bench units during steady state runs of the VTR. Abrupt changes in the operation of the VTR do not significantly affect the performance of the municipal plant. However, when the VTR is shut down to acid wash the reactor, the municipal secondary units which are trickling filters and rotating biological contactors, slough the bio-mass built up during the steady state operation when the VTR return flow is providing additional nutrients to the biological system. Sloughing results in an increase in the municipal plant effluent suspended solids and total BOD, but does not greatly affect the effluent dissolved BOD.

PROCESS CAPACITY

The Longmont VTR has a 25.4 cm (10") diameter outside reactor tube which with the 17.8 cm (7") and 12.7 cm (5") concentric tubes form a reaction chamber with a total volume of about 41.6 cubic meters (11,000 gallons). About 60% of this total volume or 25 cubic meters (6600 gallons) is in the reaction zone where the temperatures are high enough for wet oxidation to proceed. At a diluted sludge flow design rate of 0.45 cubic meters per hour (120 gallons per minute) and a gas volume fraction in the two-phase stream equal to 50%, the residence time in the oxidation zone is about 45-50 minutes,taking into consideration the compression of the gas phase.

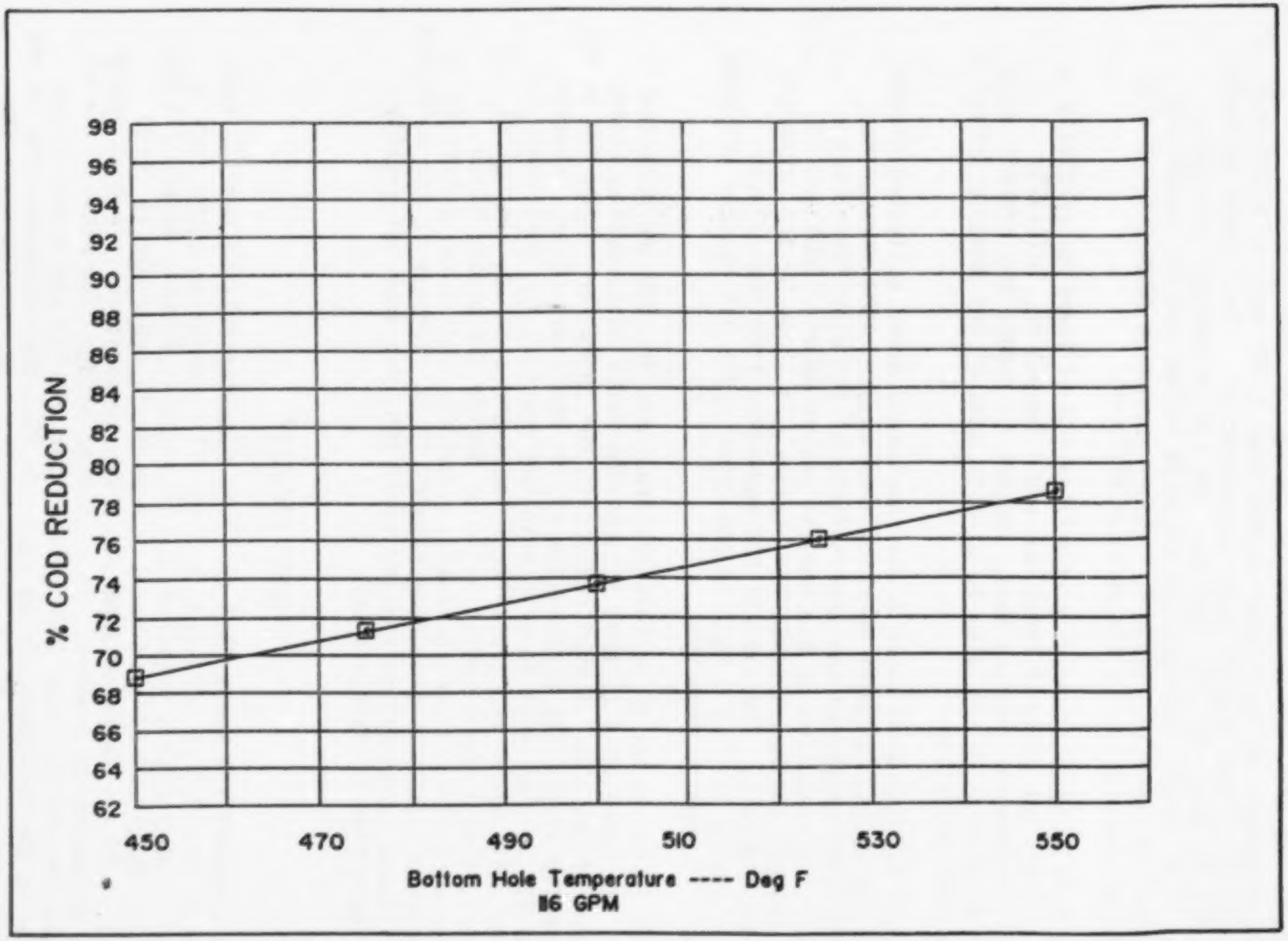

Figure 4. Effect of BHT on COD reduction.

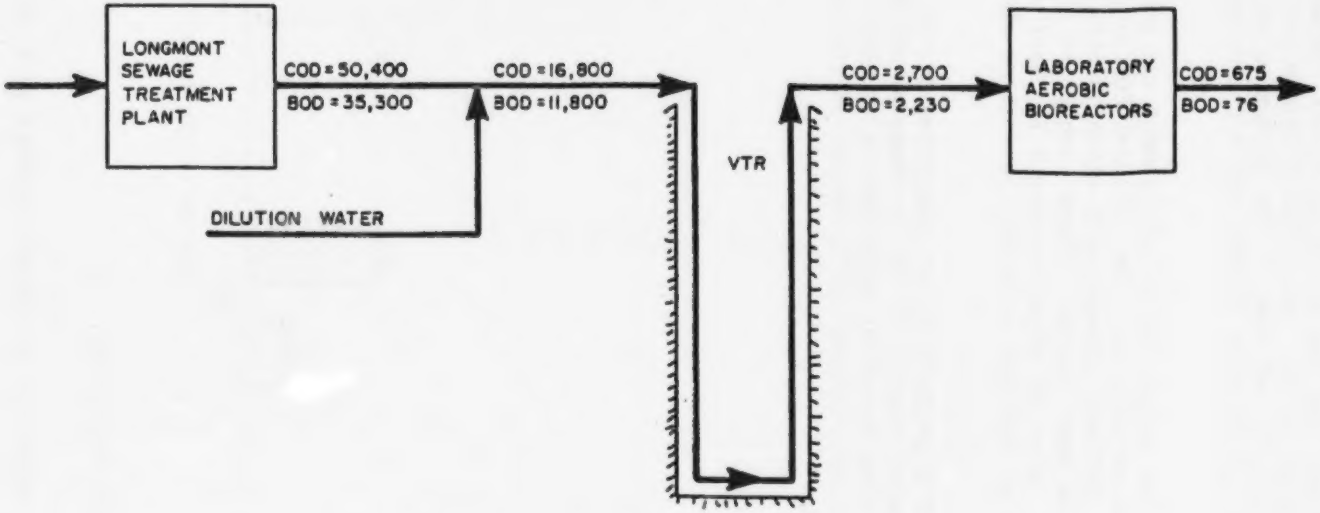
LONGMONT SEWAGE TREATMENT PLANT
COD = 50,400
BOD = 35,300
DILUTION WATER
COD = 16,800
BOD = 11,800
VTR
COD = 2,700
BOD = 2,230
LABORATORY AEROBIC BIOREACTORS
COD = 675
BOD = 76

Figure 5. Bench scale completely mixed activated sludge COD and BOD removal (mg/L).

Using air as the oxygen source, the Longmont VTR has normally treated 4500 kilograms (10,000 pounds) per day of COD or 4100 kilograms (9,100 pounds) per day of dry sludge solids. At a 4500 kilogram per day rate, it was necessary to continuously add heat to the system to overcome heat losses to the rock and to replace heat flushed out with the reactor effluent.

The equipment to provide oxygen for air enrichment was added in October 1984. With oxygen-enriched air, the influent COD strength can be significantly increased. Influent streams with strength ranging from 20,000-45,000 mg/l COD have been treated. These compare to the 10,000 mg/l COD using air alone. With the higher strength influent more heat is generated per unit volume of influent oxidized, permitting the system to operate autogenically.

Figure 6 shows VerTech's projections of capacities of two different sized reactors. The Longmont reactor is 10" in diameter and the capacities indicated are from operating experience with that facility. The capacities of the 6.3 cubic meters (16") reactor is the result of model analysis by VerTech.

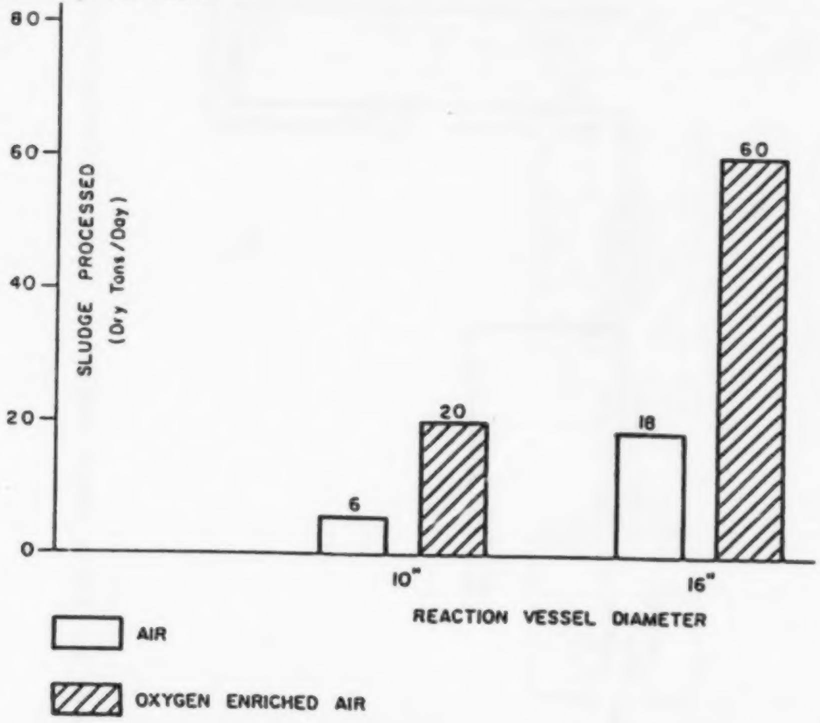

Figure 6. Capacity vs diameter effect with oxygen.

One of the early concerns was that the Longmont reactor might prove to be too small to serve the total sludge disposal needs of the Longmont Wastewater Treatment Plant. Indeed, until the oxygen-enrichment equipment was added this appeared to be the case. The wastewater treatment plant was producing sludge in amounts up to 4000 kilograms (8800 pounds) per day of COD prior to the start up of the VTR. The return flow from the VTR increased the wastewater plant sludge production by 25-30%. Sludge production for the twelve months that the VTR has been in operation has averaged 4990 kilograms (11000 pounds) per day of COD and was nearly 6350 kilograms (14,000 pounds) per day for the entire month of April 1985. The maximum loading rate on the VTR with air only was 6530 kilograms (14,400 pounds) per day of COD. This would about handle the present City plant needs but would not allow for any growth. Growth in the City is occurring at a 6% annual rate.

With oxygen-enrichment of the air supply the reactor has been operated with loads of 13600-20400 kilograms (30,000-45,000 pounds) per day of COD. Autogenic operation has been sustained at the lower loading rate but that is still more than double the present sludge production of the City wastewater treatment plant. The Longmont VTR which was initially feared to be too small, is actually too large when operated with oxygen-enriched air.

An operational solution to this pleasant problem has been to accumulate sludge for several days and then run the reactor for several days until the sludge supply is depleted.

To operate the reactor at the higher loading rates for a short time, VerTech has hauled sludge by tank trucks from two nearby municipal wastewater treatment plants.

COST OF OPERATION

Before autogenic operation was achieved, the largest operating cost of the VTR was the cost of fuel to maintain reactor temperature. With autogenic operation one of the largest operating costs for the VTR is the periodic nitric acid wash of the reactor for scale control. The buildup of scale produces an increased roughness of the pipe walls which causes greater friction losses, which in turn requires increased pumping pressures to maintain a given flow rate through the VTR. The scale does not appear to significantly reduce the cross section area of the flow path and acid washing restores lower pumping pressures. Acid washing to date has been performed after about 200,000 kilograms (220 tons) of COD processed.

Under present conditions at the Longmont wastewater treatment plant, which has very shallow final clarifiers, the wastewater plant operators have to feed aluminum sulfate as a coagulant aid to avoid violation of their plant effluent limitations. The aluminum ends up in the plant sludge. Part of the scale build up in the sludge reactor is aluminum phosphate and aluminum silicate.

Improvements now under construction at the wastewater treatment plant will eliminate the need to feed aluminum sulfate. This is expected to reduce the reactor scaling problem. If in fact it does, the cost of acid washing the reactor for scale control will be reduced.

Some of the pumping pressure build-up which was first thought to be totally the result of scaling has been found to be partially due to organic fouling which does reduce the cross-sectional areas in the top section of the reactor downcomer. Hydraulic backflushing of the reactor without acid has produced reductions in the pressure build-up by as much as 80 psi. If the pumping pressure build-up can be even partially controlled by simple back flushing, the frequency and cost of acid washing will be further reduced.

The cost of operation of the Longmont facility under steady state autogenic operating conditions is not yet available; however, VerTech is very optimistic that they can achieve operating costs of $200 or less per dry ton of solids treated. The VTR will be quite competitive with conventional methods of municipal sludge disposal if this estimated operating cost is confirmed.

The capital cost of the Longmont VTR is not a good measure of what the commercial price of similar units will be. It is a prototype designed to demonstrate the technology and operability of the process. Capital cost estimates will be included in the final report prepared for the City and the USEPA.

SUMMARY

The USEPA funded operation of the Longmont facility is essentially complete; however, VerTech plans to continue their operation and study at Longmont and we will continue to receive data. What we know at this time is:

- The Longmont VTR is a technical success. Municipal sludge is wet oxidized; more than 75% of the COD is destroyed in the sludge feed.
- The VTR effluent contains organic acids which may be returned to and be readily treated through the municipal wastewater plant secondary treatment units.

- The ash is easily separated and concentrated by gravity settling and may be disposed of by a number of acceptable methods because it is essentially inert.
- The Longmont VTR with oxygen-enriched air operates autogenically. Such operation is essential for the process to be cost competitive with other sludge disposal methods.
- The Longmont VTR with oxygen-enriched air can accept the total sludge production of the Longmont municipal wastewater treatment plant with substantial reserve capacity for growth of the City.
- Operating costs and capital costs are still being analyzed; however, VerTech is projecting operating costs at Longmont of $200 per ton of dry solids processed.

ACKNOWLEDGEMENT

The author has been in constant communication with the Engineering Staff of VerTech Treatment Systems and with Howard Delaney of the City of Longmont during the preparation of this paper. Their generous sharing of their time and knowledge is gratefully acknowledged.

MUNICIPAL WASTEWATER TREATMENT USING THE CAPTOR PROCESS

by

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Water Engineering Research Laboratory
Cincinnati, Ohio 45268

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

Prepared for Presentation at:

Tenth United States/Japan Conference
on Sewage Treatment Technology

October 17-18, 1985
Cincinnati Ohio

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ABSTRACT

The CAPTOR process is a variation of the activated sludge process in which small porous polyurethane pads are added to the reactor to provide surface area for biomass colonization. Effluent screens retain the pads within the reactors. The pads are periodically cleaned by air-lifting them onto a conveyor where they pass through rollers providing a tight compression. Cleaned pads are returned to the reactor and a seperate waste sludge stream is produced. This patented system is marketed by Simon-Hartley Ltd. and originated from studies at the University of Manchester, England. It is under evaluation on municipal wastewaters in Freehold and Stevenage, England and the U.S.EPA Test and Evaluation Facility. The original optimistic claims for process performance and advantages have not been realized to date. A pilot study of CAPTOR at Downingtown, PA eliminated the system from consideration as an upgrading approach there. The system at Freehold has undergone several modifications to obtain more uniform pad distribution and mixing and thusfar the effluent quality has not been near the original projections. Previous CAPTOR results reported in the literature are based on settled effluent samples whereas no effluent settling is envisioned for most of the applications where the process has been claimed to be advantageous; original literature references do not mention that samples were settled prior to analysis. Thusfar results at the EPA facility have not confirmed either an enhancement in oxygen transfer or process performance comparable to original projections for Freehold. While the concept underlying the CAPTOR system is quite attractive, much remains to be learned to make a realistic assessment of it's potential usefulness in wastewater treatment.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

CAPTOR DESCRIPTION

The CAPTOR process is a fixed film modification of the activated sludge process. The trade mark CAPTOR is derived from Captivated Sludge Process. The process is patented (1) and is marketed by Simon-Hartley Ltd. of Manchester, England. The U.S. representative is Ashbrook-Simon-Hartley in Houston, TX.

The CAPTOR process contains a number of novel features. Polyurethane foam support particles (25 mm x 25 mm x 12 mm) are added to activated sludge tankage to provide surface area for biomass colonization. The foam pads have a porosity of 97 percent and a specific gravity slightly greater than water. Forty pads/L is a design value considered reasonable to allow for adequate pad circulation and provide sufficient surface area for biomass growth. At 40 pads/L, 33 percent of the tank volume is occupied by support pads. Because of the high pad porosity, the actual reduction in available liquid volume is only 1 percent. The pads are manufactured by a thermal process with a pore size specification of about 0.7 mm.

Screens are utilized to prevent the support pads from exiting with the reactor effluent. Currently screens are constructed with 6 mm mesh with 60 to 70 cu m/hr/sq m considered an acceptable liquid effluent flux rate.

Excess biomass production within the pads is removed by cleaning as illustrated in Figure 1. The pads are air-lifted onto a conveyor where they are first subjected to an initial squeeze to remove water that does not freely drain from the pads. This pre-squeeze may use one or more rollers and is intended to remove excess water but not the attached biomass. Finally the pads are tightly compressed by the squeeze rollers and the cleaned pads are returned to the aeration system. A concentrated waste sludge stream is also produced.

CAPTOR ORIGIN

The CAPTOR process is an extension of studies with biomass support particles which were conducted by the University of Manchester. A number of biomass support particles were evaluated by Atkinson et al., (2,3) for use in industrial fermentation processes. In the initial studies, the most satisfactory particles were prepared from a single strand of narrow gauge steel wire and crushed into 6 mm spheres of about 80 percent porosity. Studies were expanded to include polypropylene toroids and reticulated polyester foams of 25 x 25 x 10 mm and 30 pores per inch (4).

Walker and Austin (5) described the use of both polypropylene toroids and polypropylene pads in pilot studies using a narrow 0.6 cu m column fed settled sewage and aerated with a single diffuser. Studies over a 4-month period with the 5.3 cm toroids with 92 percent porosity showed that the entrapped biomass became progressively more mineralised towards the pad center. The compromises accepted for ease of manufacture yielded a

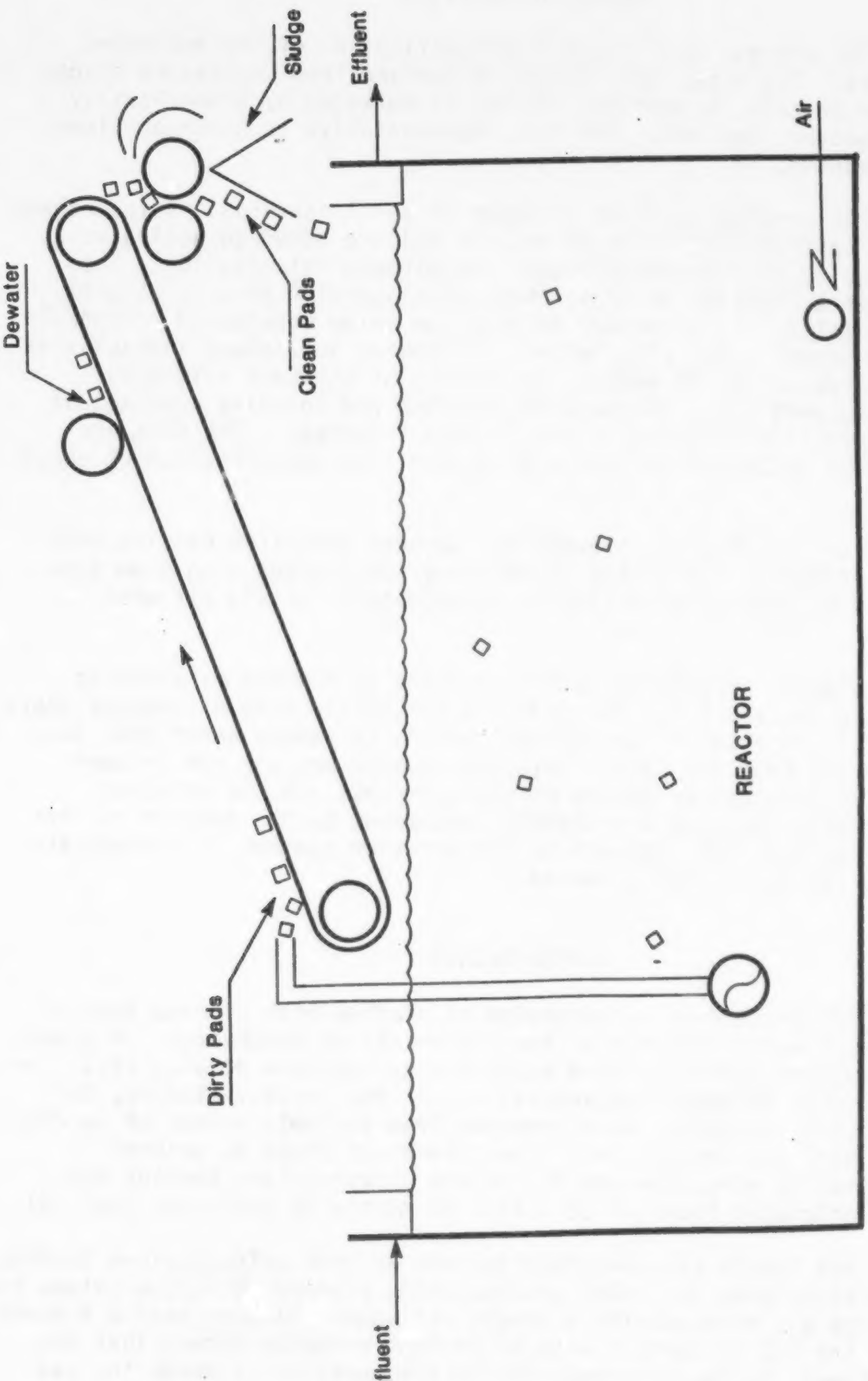
Sludge
Effluent
Air
Dewater
Clean Pads
Dirty Pads
REACTOR
Influent

Figure 1. CAPTOR System Principles

disproportionately large amount of anaerobic sludge inside the toroids requiring very low food:mass ratios to give good BOD removal or nitrification. The foam pads were desirable because they could be removed for cleaning by squeezing and it was indicated that a cleaning device was designed to yield surplus sludge at 4 to 6 percent solids. A month of operation at a 70-minute hydraulic retention time was indicated to show 85 percent total BOD removal and 92 percent soluble BOD removal. It was also stated that laboratory trials with a scaled-down CAPTOR unit had shown that 96 percent BOD removal could occur within 24 hours on an industrial waste with a BOD of up to 6000 mg/l and suspended solids of around 4000 mg/l. A 12.5 cu m system was designed and was expected to go on stream in early 1981.

By 1981, Simon-Hartley Ltd. was promoting CAPTOR as a process ideally suited for pretreatment of strong industrial wastes prior to sewer discharge (6). A typical installation would consist of aerated reactor, pad cleaner and sludge holding tank. No secondary settlement or biomass recycle was required. Waste sludge was claimed to contain up to 6 percent solids and biomass concentrations were stated to be equivalent to 10 to 15 grams/L.

INITIAL U.S. EPA INVOLVEMENT WITH CAPTOR

In February 1983, a technology exchange meeting was held in Cincinnati with representatives from the Water Research Center (WRC) in Great Britain, Environment Canada and the U.S. EPA. CAPTOR was one of several systems discussed. Simon-Hartley, the WRC, the Severn-Trent Water Authority and the Swiss Federal Office for Environmental Protection were initiating an evaluation of CAPTOR at Freehold, England. Financial support from both the U.S. EPA and Environment Canada was solicited by the WRC.

The 18,700 cu m/day (5 mgd) plant at Freehold contains five parallel aeration basins and two final clarifiers (7). The plant could not achieve an acceptable degree of nitrification for more than three months per year and upgrading to acheive year-round nitrification was required. It was proposed that this could be achieved by modifying the first 25 percent of each of the five aeration lanes to the CAPTOR process. Prior to a complete plant conversion, however, it was proposed to modify just two of the existing lanes to include CAPTOR and to retain two lanes as an activated sludge control. Each system was to receive 50 percent of the primary effluent flow. An economic evaluation indicated that the approach with CAPTOR was more cost effective than the addition of nitrifying trickling filters provided the rate of pad replacement was not in excess of about 20 percent per annum. This economic analysis took no credit for claimed improvements in aeration efficiency with CAPTOR.

Preliminary studies in a 0.6 cu m column (7) indicated that the presence of the pads significantly increased the oxygen transfer efficiency of both fine and coarse bubble diffusers with a 5 to 6 fold increase in K_La being reported for fine bubble aeration. Increased oxygen transfer efficiencies (OTE's) would be an additional economic benefit for CAPTOR.

A summary of process operation and projected performance for the Freehold pilot study is presented in Table 1. The projected CAPTOR BOD removal was indicated to be based on data collected from three seperate studies where BOD removal was related to volumetric loading by

$$\text{Percent BOD Removal} = 98\ e^{-0.12(\text{Volumetric Loading, kg/cu m/day})}$$

or to biomass loading by

$$\text{Percent BOD Removal} = 105\ e^{-0.67(\text{Biomass Loading, kg BOD/kg biomass/day})}$$

However it was not made clear that these relationships were only applicable to CAPTOR effluent samples which had been settled for one hour (8) and therefore were not appropriate for calculating the organic load to a CAPTOR system discharging directly into a subsequent biological system.

TABLE 1. DESIGN VALUES FOR FREEHOLD, ENGLAND CAPTOR EVALUATION

Average CAPTOR Flow	9350 cu m/day (2.5 mgd)
Primary Effluent BOD	144 mg/l
F:M (CAPTOR only)	0.56 kg BOD/kg pad MLSS
Pad Concentration	40/L
Pad Solids	180 mg/pad
Equivalent MLSS	7200 mg/l
BOD Removal (CAPTOR only)	75 % (70 % winter, 80 % summer)
CAPTOR HRT	0.84 hours
Total HRT per Lane	3.4 hours
Waste Sludge Concentration	5 %
OTE	10 %

Based on the potential benefits which were presented for a CAPTOR system and an interest in high biomass processes, the U.S. EPA awarded a cooperative agreement to WRC in September, 1983 to cover a portion of the costs and gain access to the data at Freehold as well as construct a pilot-scale CAPTOR system at the EPA Test and Evaluation Facility (T&E) in Cincinnati, Ohio.

CAPTOR PILOT STUDIES IN THE U.S.

The first pilot study of the CAPTOR process on municipal wastewater in the U.S. was in Downingtown, Pennsylvania with the period of formal data collection from 16 January thru early May, 1984. The study was undertaken by the city at their expense because they were required to upgrade their plant to achieve nitrification, and it was felt that an approach similar to that proposed for Freehold would be cost effective. Data from this study were made available to the WERL-WRD.

The CAPTOR pilot reactor was 2.4 x 7.3 x 2.4 m in depth (8 x 24 x 8 ft) with the direction of flow parallel to the 7.3 m length. Reactor volume was 43.5 cu m (11,480 gal) providing a hydraulic residence time (HRT) of approximately 1 hour at an influent flow of 757 L/min (200 gpm). Overall pad concentration was 42 pads/L although pad density was higher at the effluent end of the reactor. Aeration and mixing were provided with 54 ceramic diffusers.

Data from various periods of operation are presented in Table 2. The influent wastewater to this plant was relatively weak with a mean influent BOD_5 concentration for the entire period of 101 mg/L. This corresponds to an applied loading of 0.84 kg BOD_5/day/kg pad MLSS at 757 L/day (200 gpm) and pad solids of 72 mg/pad. Mean filtered BOD_5 removals shown in Table 2 varied from 20 to 28 mg/L and seemed to increase somewhat as the waste strength increased. Considering only the data where the influent filtered BOD_5's were in excess of the median value of 43 mg/L, gives filterable BOD_5 removal of 28 mg/L or 43 percent and total BOD_5 removal of 31 mg/L or 24 percent. It is clear, however, that BOD removals were not strongly influenced by the range of influent and operating parameters at this site.

Pad solids did not display any consistent variation and displayed little sensitivity to the range of conditions encountered. The pad solids concentration from 7 to 28 March, when the pad cleaner was operated most frequently, were essentially the same as for 29 March to 23 April when the pad cleaner was only operated 1/6 as often. Effluent SS were consistently somewhat higher than the influent concentration reflecting excess biomass growth in the system not removed via sludge wasting.

From 1 February to 16 March, the average pad cleaner run time was 4.74 hours/day and the waste sludge concentration was measured routinely (21 measurements). Data from this time period showed an average of 75 mg/L of SS leaving in the process effluent. The pad cleaner produced waste sludge flow with a mean solids concentration of 10700 mg/L. This is equivalent to an additional 21 mg/L of SS that were removed via wasting rather than in the process effluent. The sum of these values (96 mg/L) is higher than expected indicating some variation in the waste sludge flow (a constant 7.57 L/min (2 gpm) assumed) and/or some error in the solids measurements. Nonetheless, this analysis shows that even during the period of most extensive pad cleaner operation at this site approximately 78 percent of the combined influent SS plus biomass growth were escaping in the process effluent. The mean 44 mg/pad solids removal and waste sludge estimates suggest that about 30 percent of the pads were cleaned each day under these operating conditions. Boyle and Wallace (8) reported 8 to 9 percent pads cleaned/hour corresponding to 38 to 43 percent of the pads cleaned/day.

TABLE 2. CAPTOR PERFORMANCE AT DOWNINGTOWN, PA

	16 Jan - 6 May[a]		1 Feb -22 Apr[b]		7 Mar - 28 Mar[c]		sBOD > 43mg/1[d]		29 Mar -23 Apr[e]	
	No. Samples	Mean Value	No. Samples	Mean Value	No. Samples	Mean Value	No. Samples	Mean Value	No. Samples	Mean Value
Total BOD_5, mg/l										
Influent	91	101	72	93	21	84	45	127	23	86
Effluent	92	78	72	72	21	70	45	96	23	61
Filtered BOD_5, mg/l										
Influent	89	48	70	43	21	37	45	65	23	40
Effluent	92	26	72	22	21	17	45	37	23	17
Suspended Solids, mg/l										
Influent	97	66	78	62	22	64	45	74	26	56
Effluent	98	73	78	68	22	77	45	81	26	60
Pad Solids, mg/pad										
Before Cleaning	78	72	58	71	15	66	37	77	14	63
After Cleaning	79	35	59	34	14	33	37	37	14	37
Waste Sludge, mg/l	30	9980	21	10700[f]	--	--	--	--	--	--

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[a] Entire period of data collection
[b] Period of steady 757 L/min (200 gpm) influent flow
[c] Mean pad cleaner run time of 6 hours/day; DO 2 to 5 mg/l
[d] Data when filtered influent BOD_5 > median value of 43 mg/l
[e] Mean pad cleaner run time of 0.9 hours/day
[f] Last measurement was March 17, 1984

Based on the results of this pilot study, a decision was made to exclude the CAPTOR process from further consideration for upgrading at the Downingtown facility.

The second pilot study of the CAPTOR process on a municipal wastewater in the U.S. was at Marion, Illinois in the Spring of 1984. This pilot unit also consisted of a first-stage CAPTOR unit fed primary effluent and discharging directly into a second-stage activated sludge unit. Sodium aluminate and polymer were added to the raw wastewater resulting in a primary effluent BOD of roughly 75 mg/L (8). Pad biomass solids during the entire period of operation ranged from 45 to 70 mg/pad. The data available for process characterization are quite limited and not of a consistent high quality. Shortly after the study began, the State of Illinois EPA decided that an innovative process such as CAPTOR was unacceptable regardless of the pilot study results.

CAPTOR STUDY IN FREEHOLD ENGLAND

During the same period of disappointing CAPTOR pilot plant results in the U.S., a paper presented by Cooper et al., (9) described selected results from the study at Freehold. It was reported that a number of unsteady-state step-change tests has shown that the k_{La} values obtained in CAPTOR were higher by 2 to 3 fold than in the conventional activated sludge system with fine bubble aeration. Oxygen utilization (OTE's) in the CAPTOR system with 27 pads/L was estimated at 18 percent. Data from various experimental runs are summarized in Table 3. BOD removals varied between 45 to 60 percent and it was indicated that 70+ percent BOD removal was still an achievable objective. Walker and Austin (10) summarized plant performance at Freehold by indicating that a feed BOD of 140 mg/L yielded CAPTOR effluent BOD's of 50 to 60 mg/L in a 45-minute retention time corresponding to mean BOD removals of 61 percent.

TABLE 3. CAPTOR TEST RESULTS AT FREEHOLD, ENGLAND REPORTED BY COOPER ET AL. (9)

	22 Nov - 31 Dec, 1982	1 June - 7 July, 1983	15 Sept - 4 Nov, 1983
Pad Concentration, pads/L	27	18	28
Pad Biomass Concentration, mg/pad	143	260	162
HRT in CAPTOR, min	43	46	47
Influent BOD, mg/l	114	142	136
CAPTOR Effluent BOD, mg/L	45	78	74
Influent SS, mg/L	101	122	144
CAPTOR Effluent SS, mg/L	81	90	97

It was not until November, 1984 that the U.S. EPA in Cincinnati began receiving copies of the CAPTOR system progress reports which had been prepared for the CAPTOR Steering Committee. These reports contain tabulated data and brief summaries of results from Freehold as well as results from a number of pilot scale studies at Stevenage, England. These reports provided further evidence that there were problems with CAPTOR operation at Freehold as indicated by Boyle (11). Boyle and Wallace had visited the Freehold facility in the summer of 1984 as part of their assignment under U.S. EPA contract to evaluate porous biomass support systems (8).

The Freehold CAPTOR system has experienced a variety of problems including pad cleaner reliability and pad mixing. There was severe pad maldistribution and typically one would find pad concentrations of 5 to 10 pads/L at the inlet end and 40 to 60 pads/L at the outlet end (12). There were frequent problems with large "rafts" of pads floating on the tank surface. Various modifications with different diffuser configurations were made in an attempt to overcome the mixing problem. At the beginning of 1984 a decision was made to temporarily switch the major effort from full-scale experimentation at Freehold to smaller scale development at Stevenage (13).

Given all of the modifications and operating problems at Freehold, it is difficult to identify periods of operation in the progress reports which would represent stable, trouble-free operation. Table 4 summarizes selected performance data in the progress reports for periods which appeared to have no extreme performance problems. These data show BOD removals of 20 percent or less and no suspended solids removal. These results are in marked contrast to those previously reported for this same facility by Cooper et al. (9) and Walker and Austin (10). In particular, the results from June, 1984, which reflect an additional year of plant modification compared to the June/July data in Table 3, show BOD removals of only 8 and 15 percent.

TABLE 4. CAPTOR PERFORMANCE AT FREEHOLD, ENGLAND

	7 Nov-15 Dec, 83	March, 84	June, 84	
Unit Designation	C2[a]	C1[b]	C1	C2
Influent Flow, cu m/day	261	218	223	223
HRT, min	38	46	45	45
Influent BOD, mg/L	156(16)[c]	120(10)	155(9)	155(9)
Effluent BOD, mg/L	123(16)	115(12)	133(9)	144(9)
Influent SS, mg/L	140(22)	105(19)	105(19)	105(19)
Effluent SS, mg/L	149(19)	112(21)	105(18)	136(18)
Pad Biomass, mg/pad	151(29)	137(22)	114(17)	161(17)

[a] 17 pads/L
[b] 28 pads/L
[c] Number of Samples in Parenthesis

Major modifications have been made to the facility in Freehold within the last few months and data generated in 1985 will represent a hydraulic and staging configuration of the CAPTOR system substantially different than previously utilized.

U.S. EPA CAPTOR PILOT PLANT STUDIES

Three pilot plant reactors were constructed for CAPTOR evaluation at the EPA T&E facility in Cincinnati. The reactors have a liquid volume of approximately 15.3 cu m (540 cu ft) and can be operated in series or parallel. Feed is primary effluent from the Mill Creek Wastewater Treatment Plant. Aeration is provided by EDI Reef fine bubble diffusers. Because of small differences in tank geometry, 6 diffusers are located in one tank whereas the remaining two contain 5 diffusers each. The diffusers are located down one wall of the tank (beneath the effluent screens) and produce a strong spiral flow in the completely mixed reactors. A schematic of Tank A is shown in Figure 2.

OXYGEN TRANSFER EFFICIENCY

Because of the reported large enhancement in oxygen transfer efficiencies with a CAPTOR system (7,9,10), the pilot studies were designed to confirm and quantify this effect. Specifically, clean water OTE's with no pads added, OTE's with a mixture of recycle activated sludge and primary effluent (no pads), and OTE's in the CAPTOR system itself have all been determined.

Clean water efficiencies were determined in accordance with the ASCE Standard Procedure (14). Three DO probes with one ml thick membranes and strip chart recorders were used for each determination. Tap water from the City of Cincinnati was used and changed as needed to remain below the allowable limit for total dissolved solids. Data were analyzed by the non-linear regression method. The mean clean water OTE's in the three reactors determined from the results of duplicate tests at each air flow rate are shown in Table 5.

TABLE 5. CLEAN WATER TRANSFER EFFICIENCIES IN CAPTOR SYSTEM REACTORS

Date Dec. 1984	Tank No.	Mean Air Flow scfm	Water Temperature °C	OTE 20°C and 0 DO %	Diffusers Per Tank
11	A	25.5	17.5	15.0	6
12	A	63.8	17.5	15.9	6
13	A	45.3	18.0	16.3	6
16	B	25.6	16.8	14.5	5
16	B	64.1	17.0	14.8	5
20	B	45.3	17.0	15.6	5

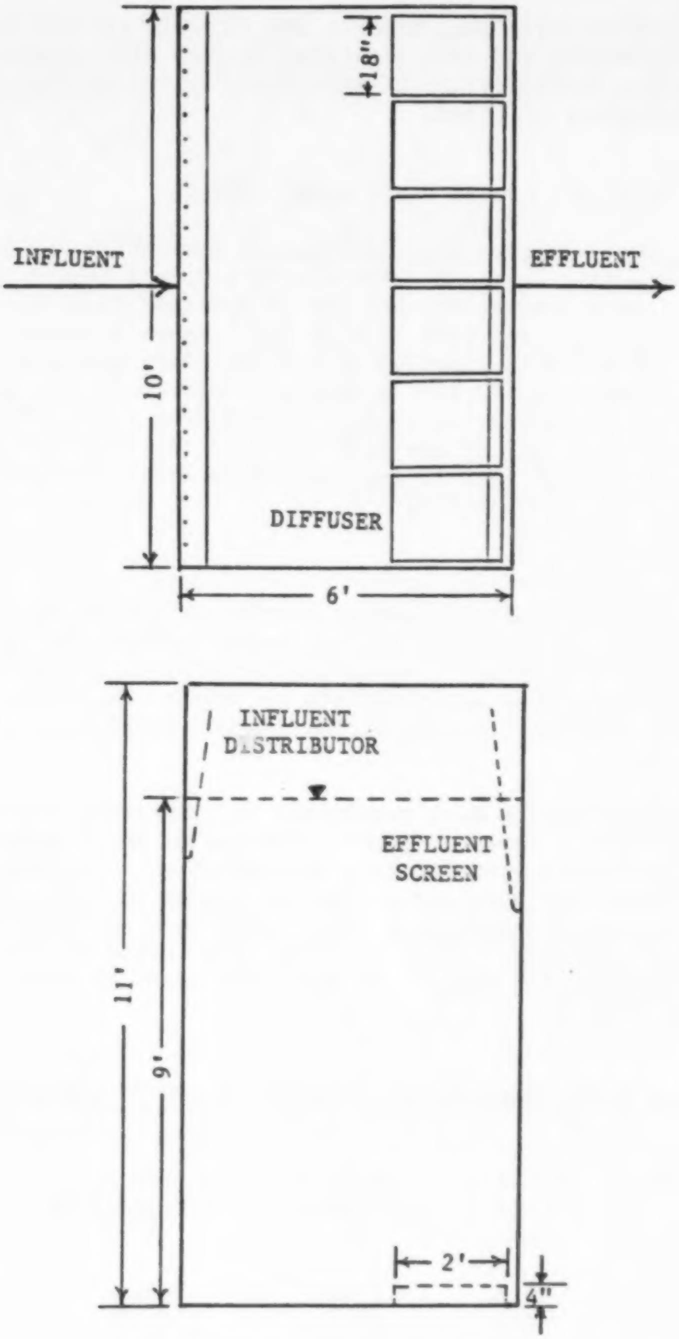
INFLUENT
EFFLUENT
18"
10'
DIFFUSER
6'
INFLUENT DISTRIBUTOR
EFFLUENT SCREEN
11'
9'
2'
4"

Figure 2. Schematic Diagram of Reactor No. A. at the T&E Facility

Dirty water OTE's were measured by offgas analysis (15) using an analyzer manufactured by Ewing Engineers and a 0.9 x 1.2 m (3 x 4 ft) hood for gas capture. From 29 January to 1 February, 1985 the pilot system was operated with series flow through the three reactors. Primary effluent and return activated sludge flows were approximately 170 and 57 L/min (45 and 15 gpm), respectively providing an HRT of about 67 minutes per reactor. Parallel flow with 95 L/min (25 gpm) primary and 57 L/min (15 gpm) recycle was used for the offgas testing on 12 February resulting in an increase in HRT to 100 minutes in Tanks 2 and 3. From 5 February to 10 February there was so much foam on the reactor surfaces that it rose above the 0.6 m (2 ft) of freeboard and overflowed the top of the tanks. Under these circumstances it was not possible to use the offgas analyzer. The offgas results are presented in Table 6 where they are grouped by stage of treatment. The alpha values in the lead stage range from 0.20 to 0.51. Except for the data from 11 February, the second and third stages exhibit the typical increases in alpha that one would expect from fine bubble systems operating in a reactor-in-series configuration where most of the soluble BOD removal was occurring in the lead stage. Whatever entered the sewer system and caused the severe foaming problem, resulted in significant alpha depression on 11 to 12 February compared to the values obtained prior to that time.

TABLE 6. DIRTY WATER OFFGAS ANALYSIS RESULTS GROUPED BY STAGE OF TREATMENT

Reactor No.	Date	Air Flow scfm	DO mg/L	Alpha[a]	% OTE at 20°C and 0 DO
		FIRST STAGE OF TREATMENT			
A	29/1	52.4	0.9	0.36	5.64
A	30/1	56.8	2.0	0.30	4.70
A	4/2	35.9	1.8	0.51	8.04
A	11/2	24.9	1.7	0.20	3.17
B	12/2	11.3	2.9	0.25	3.70
C	12/2	11.1	4.7	0.40	5.94
		SECOND STAGE OF TREATMENT			
B	30/1	14.9	2.0	0.56	8.33
B	1/2	14.5	3.7	0.66	9.92
B	4/2	14.7	3.4	0.71	10.6
B	11/2	11.6	2.5	0.30	4.54
		THIRD STAGE OF TREATMENT			
C	31/1	12.6	3.7	0.69	10.3
C	4/2	13.0	3.4	0.77	11.5
C	11/2	10.3	2.7	0.33	4.92

[a] Based on mean clean water OTE for this tank

CAPTOR PERFORMANCE AT THE T&E FACILITY

Following pad addition and system startup, the first period of steady state CAPTOR data collection was from 19 May to 23 June, 1985. During this period the three reactors were operated in parallel with influent flows of 95 L/min (25 gpm) each. The pad cleaners were operated to clean 1/5 the number of pads in each reactor per day. Inlet air flows of 2.1, 1.4 and 0.71 cu m/min (75, 50 and 25 scfm) were provided to Tanks A, B and C, respectively. The impact on daily mean DO levels is shown in Figure 3. Offgas testing was performed on two occassions with the results indicated in Table 7. Based on comparing the first stage OTE's in Table 6 with the values in Table 7, there is no evidence thusfar of enhanced OTE's with this system. Several more determinations are required to confirm the lack of enhanced oxygen transfer.

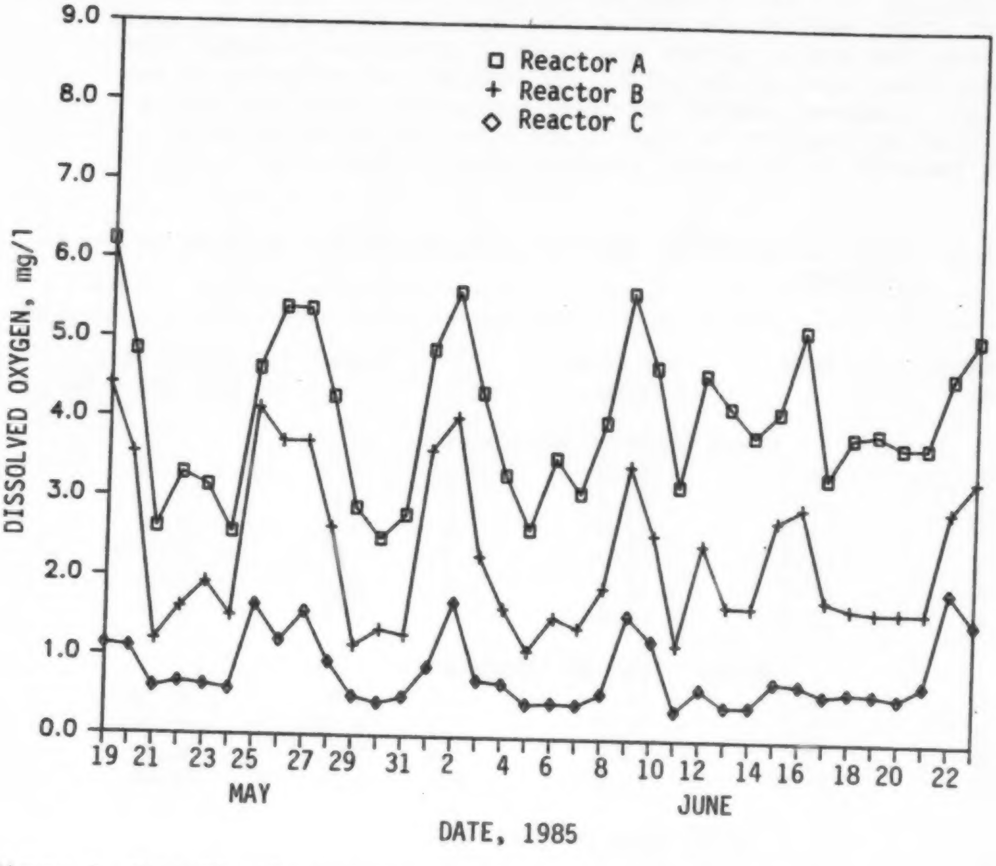

Figure 3. Mean Dissolved Oxygen Levels in the CAPTOR Systems

TABLE 7. OTE'S FOR THE CAPTOR SYSTEM

Reactor No.	Date 1985	Air Flow scfm	Reactor DO, mg/l	% OTE 0 DO and 20°C
A	12/6	72.0	3.1	3.67
B	18/6	50.2	1.5	4.74
C	18/6	24.8	1.0	4.99
A	1/7	74.9	5.0	7.39
B	3/7	49.8	4.0	4.93
C	3/7	24.4	1.0	3.67

Selected data from the CAPTOR systems are presented in Table 8. Although the mean DO in Reactor C was 0.8 mg/L, this system frequently operated at lower DO levels (Figure 3) and it is clear that insufficient DO was present in this system on some occassions. At the start of these studies there was considerable speculation that the low pad solids levels which had been observed in U.S. pilot studies were the result of relatively weak wastewaters and/or excessive turbulence and resulting shearing of biomass from the pads. The results in Table 8 show no correlation of air flow and pad solids levels. Furthermore, the waste strength at this facility is roughly the same as that at Freehold, England (Table 1). Where DO was not limiting, total BOD_5 removals were about 54 percent and filtered BOD_5 removals were 84 to 90 percent. The 2.7 hour HRT was 3.2 times greater than the design value for Freehold where total BOD removals of 75 percent were projected.

TABLE 8. MEAN VALUES FOR CAPTOR OPERATION AT THE T&E FACILITY FROM 19 MAY to 23 JUNE, 1985

	Influent	Reactor No. A	B	C
Total BOD_5 mg/l	144(25)[a]	68(26)	65(26)	91(26)
Filtered BOD_5, mg/l	106(26)	11(26)	17(25)	40(26)
Suspended Solids, mg/l	123(35)	154(35)	153(35)	141(35)
Pad Solids, mg/pad		73(32)	82(32)	71(32)
Dissolved Oxygen, mg/l		4.1(216)	2.3(216)	0.8(216)
Waste Sludge Concentration, %		0.64(36)	0.68(36)	0.97(35)

[a] Number of 24-hour composite samples or number of measurements

SUMMARY

The concept of porous biomass support systems such as CAPTOR offers a number of potentially attractive advantages. Other approaches with porous pad media, such as that of Linde AG of West Germany, are also under development (8). Extensive pilot studies at Stevenage and Freehold and the U.S. T&E facility in Cincinnati should provide an adequate basis to assess true process potential for CAPTOR. The potential of this system for nitrification applications also needs to be investigated.

To date CAPTOR results have been nowhere near the optimistic early projections for the process. The initial performance projections were based on settled samples from the CAPTOR reactor when, in fact, no settling occurs. Claims such as enhanced OTE's are also unverified. Fundamental issues such as the best basin configuration and diffuser arrangement are still unanswered. It is clear that much remains to be learned about the true potential and limitations of this process.

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BIOLOGICAL AERATED FILTER PERFORMANCE

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This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

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BIOLOGICAL AERATED FILTER PERFORMANCE

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ABSTRACT

The Biological Aerated Filter (BAF) system is a high rate downflow fixed film treatment process applied after primary treatment of domestic wastewaters. No secondary clarification is required after BAF treatment and the system requires about one-fifth the land area of the conventional activated sludge process. The system has been studied in an EPA Demonstration Project in Salt Lake City, Utah. The study showed that a critical operating and performance parameter for the system is the size of the media used. An effective media size of 3.4 to 4.4 mm is recommended for wastewaters with a high proportion of BOD in the particulate form and with a significant influent suspended solids concentration to minimize backwash requirements. Organic loading rates to achieve secondary treatment of domestic wastewaters may range from 3 to 4 kg BOD/m^3-day (180 to 250 lb BOD/1000 cu ft-day) depending on the wastewater temperature. The organic loading rate could be higher if the wastewater has a more soluble BOD form to allow the use of a smaller media size. Oxygen consumption rates and sludge production rates are a function of the wastewater characteristics. The oxygen consumption rate could vary from 0.6 to 1.0 kg oxygen per kg of BOD removed. The sludge production rate could vary from 0.75 to 1.1 kg per kg of BOD removed.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

INTRODUCTION

There has been considerable interest in the past few years in developing biological wastewater treatment processes that reduce reactor volume and land area requirements. These biological process designs, generally employing a fixed film treatment mechanism, include plastic media trickling filters, rotating biological contactors, the Captor process (1), fluidized bed reactors (2), the PBR system (3), the Acticontact process (4), and the Biological Aerated Filter (BAF) system (5,6). The last four of the above processes are characterized by a relatively high fixed film surface area per unit volume, a relatively high reactor biomass concentration, and mechanical oxygen dissolution methods to support the higher volumetric biological oxidation rates.

DESCRIPTION OF BAF SYSTEM

In the BAF system wastewater is passed downflow through a 1.5-1.8m bed of vitrified clay media. The effective size of the media used has ranged from 3.0 to 4.0mm. For domestic applications, primary treatment is normally required ahead of the BAF system. Pretreatment is necessary to reduce suspended solids and grease to acceptable levels. No additional clarification is used after the BAF treatment system. Oxygen is supplied by air sparging through an air header system located about 30cm. above the bottom of the media. The media is supported by an underdrain plate, and plastic nozzles are used to collect the treated water and distribute backwash water and air. BOD removal is accomplished by solids filtration and biological metabolism of soluble organics. Periodic backwashing is necessary to flush out excess biological solids and filtered solids to minimize the operating headloss. The system operation is completely automated by a microprocessor that initiates and controls a backwash sequence of air, air/water and water.

Backwashing is initiated by the microprocessor on a timed basis or by a level probe sensing a certain headloss build-up above the media. The first step of the backwash sequence involves the addition of air to the bottom of the bed at a rate of 0.9 m/min (3 scfm/ft^2) for about one minute. Then air and water are applied simultaneously with a water application rate of 0.73 m/min (189 gpm/ft^2) for about 1 minute. At this point a level probe detects the higher water level and both the air and water applications are stopped for one minute to allow the media to settle on the bed. Water is then applied again at the previous rate for about 20 seconds. The water level in the BAF cell is then high enough to cause the backwash water to flow through siphon pipes located in the cell. About two minutes elapse before the backwash water is siphoned out of the cell. This procedure is repeated four to five times for a complete backwash event. During some of the earlier operating experiences, the described backwash cycle was completed two times when the backwash was initiated by a headloss build-up and five times for a timed backwash event.

The claimed design loadings by the BAF developers to achieve secondary treatment have been in the range of 3 to 5 kgBOD/m^3-day (190-310 lbs/1000 cu.ft.-day). In January 1983 a BAF system with a nominal flow of 1893m^3/day (0.5 MGD) was started up at the South Davis Sewer Improvement District south plant near Salt Lake City under an EPA demonstration plant project. The objective of this project was to evaluate the BAF system relative to BOD and suspended solids removal, oxygen requirements, energy requirements, sludge production, backwash requirements, media characteristics, operational requirements and mechanical reliability. This paper will summarize the performance results for a one year operating period relative to the above objectives for this study.

DEMONSTRATION PLANT DESCRIPTION

BAF FACILITY

Figure 1 shows a schematic of the BAF facility. The BAF system processed a portion of the primary effluent stream at the South Davis plant that treats a total average flow of approximately 11,370 m^3/d (3 MGD) through a two stage trickling filter system. The wastewater consists of 70 to 80 percent domestic wastewater with industrial contributions originating from an oil refinery, a small tannery, and a laundry. The BAF system has two self priming centrifugal pumps rated at 2.2 and 3.7 kw (3 and 5 Hp) each to independently feed primary effluent to each of the two cells, designated East and West. Each cell is 3.7 m (12 ft) x 3.2 m (10.5 ft) and has a media depth of about 1.7m (5.7 ft). The pumping rate of each feed pump was controlled by variable frequency drive (VFD) controllers. A custom built signal generator directed a signal to the VFD controllers to vary the flow each hour to simulate diurnal flow patterns. A typical flow pattern used for each cell is shown in Figure 2 for a 1.3 peak to average (P/A) flow variation. The signal generator also controlled influent and effluent sample pumps to provide hourly flow proportioned samples that were stored in a 4°C refrigerator at the site. Propellor type flow meters were installed in the effluent lines from each cell to record the instantaneous flow rate on strip chart recorders. The accumulative flow was indicated by a digital readout on the meter.

Automatic backwashing was initiated by either a signal to the microprocessor from a level probe as the headloss built up in the BAF cell or by programming for a timed backwash cycle. The microprocessor would then shut off the appropriate feed pump and one of the two 7.5 kw (10 Hp) process air blowers, activate the 15 kw (25 Hp) backwash air blower and the 15 kw (25 Hp) backwash water pump, and then open and close the necessary valves at programmed time intervals to carry out the backwash procedure. When the backwash water clear well volume was depleted, a level probe provided a signal to open an automatic drain valve to direct process effluent to the clear well. The backwash water was collected and drained to the plant headworks. An automatic sampling system was also operated by the microprocessor, to collect a backwash solids sample during each backwash flush.

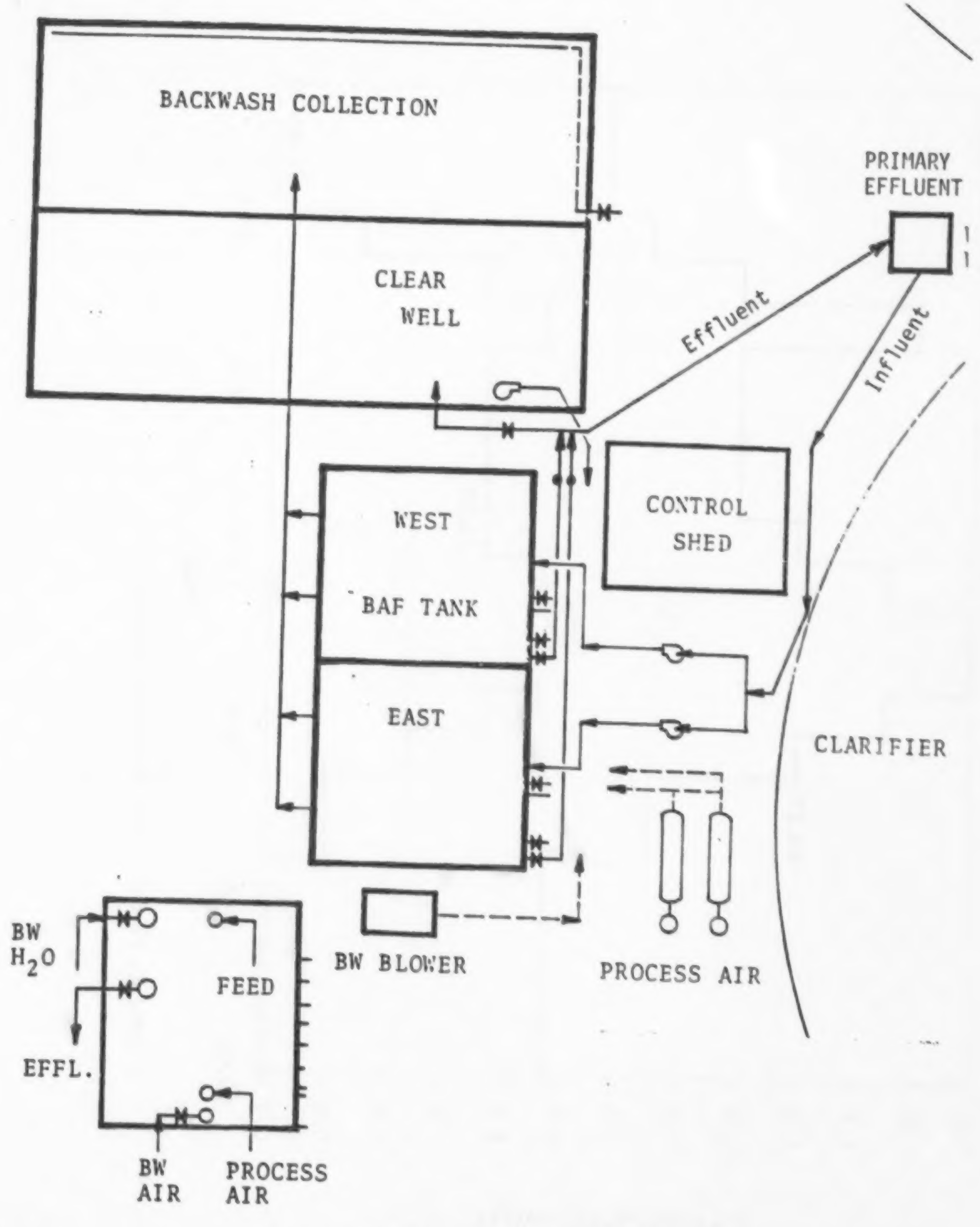
BACKWASH COLLECTION
PRIMARY EFFLUENT
CLEAR WELL
Effluent
Influent
WEST
BAF TANK
EAST
CONTROL SHED
CLARIFIER
BW H$_2$O
EFFL.
FEED
BW BLOWER
PROCESS AIR
BW AIR
PROCESS AIR

Figure 1. Biological aerated filter demonstration plant.

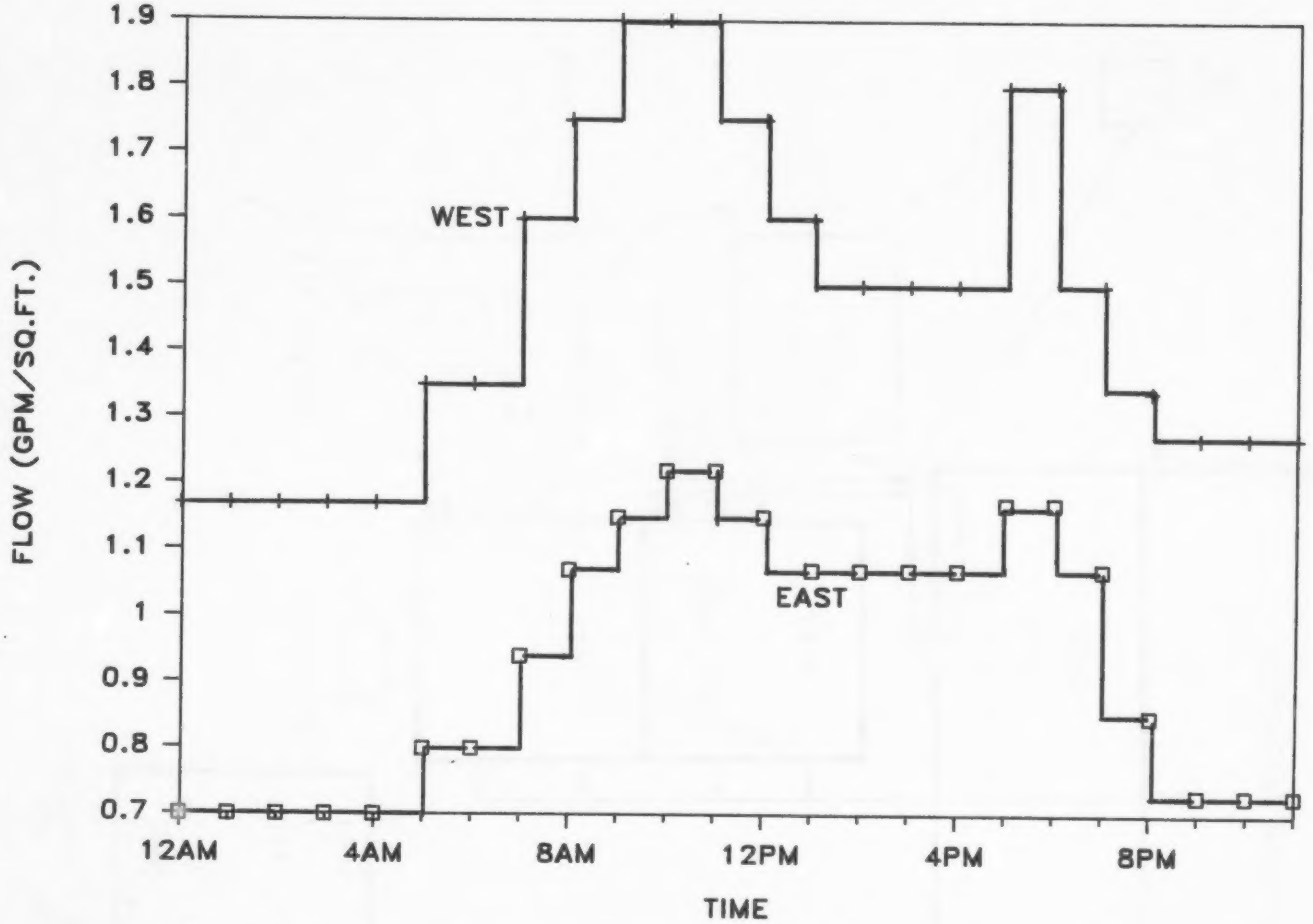

Figure 2. Diurnal flow variation pattern.

OPERATING CONDITIONS AND DATA COLLECTION

As shown in Table 1, the West cell was operated with a much higher hydraulic application rate than the East cell. The original operating objective was to operate at about a 5 kg BOD/m^3-day (310 lb/1000 cu ft-day) organic loading in the West cell to meet 30/30 mg/l BOD and total suspended solids (TSS) effluent concentrations. A 3 kg BOD/m^3-day (190 lb/1000 cu ft-day) operating load was set for the East cell to meet 10-10 mg/l BOD and TSS effluent concentrations. The actual loadings achieved were generally about 20 percent lower because infiltration to the plant was higher than normal during this study period due to record breaking precipitation in the Salt Lake City area. The average hydraulic application rate was limited under these conditions by allowable peak flow hydraulic application rate that would not result in a rapid headloss build-up.

TABLE 1. BAF OPERATING CONDITIONS

Date	Hydraulic Application Rate (m/hr) West Cell	Hydraulic Application Rate (m/hr) East Cell	Peak to Average Flow West Cell	Peak to Average Flow East Cell
June-October 1983	3.9	2.4	1.3	1.0
October-November 1983	3.9	2.4	1.0	1.0
December-January 1984	2.4	2.4	1.0	1.0
February 1984	3.4	2.4	1.4	1.3
March-June 1984 NEW MEDIA	2.4	1.2	1.0/1.5	1.0/1.5

Empty Bed Volume Detention Time: 26 min. at 3.9 m/hr.
43 min. at 2.4 m/hr.

During October and November 1983, a constant flow operation was used, because of problems encountered with excessive headloss build up and a high backwash frequency requirement. This appeared to be caused by two successive problems. During the last week of September and early October, one of the plants digesters was cleaned, resulting in primary effluent TSS concentrations in the range of 200-300 mg/l. This caused a rapid headloss build-up and shortened the BAF operating time between backwashes. The second problem causing a high backwash frequency appeared to be due to the presence of a fiber or hair-like material in the influent wastewater. Thin fibers, which were about 2-9 cm (0.8 to 3.5 in) in length, were collected on the top of the BAF cell media and caused a formation of a mat of fiber and solids. The fiber problem diminished some time during December. The hydraulic application rate for both cells was similar in December since different backwash techniques were used to attempt to minimize the backwash frequency.

During January, the hydraulic loading to the West cell was not increased due to a reduced BOD removal efficiency. This was affected by both a much lower wastewater temperature and higher wastewater strength. As the wastewater temperature increased in February, the hydraulic application rate to the West cell was increased. The diurnal flow variation was also resumed in both cells.

In early March 1984, the media in both cells was changed to one with a larger effective size to determine if this would result in a lower backwash frequency. A lower treatment efficiency was expected with the larger media since the biofilm surface area per unit volume was decreased. The previous media had an effective size of 2.8 mm and a 1.6 uniformity coefficient. The replaced West cell media had an effective size of 4.4 mm with a 1.6 uniformity coefficient, and the replaced East cell media effective size was 3.4 mm with a 1.5 uniformity coefficient. Based on the effective size, the new media in the West cell had about 63 percent of the surface area per unit volume as the original media.

The 24 hour flow proportioned composite samples were analyzed for total and soluble BOD_5 and COD and total and volatile suspended solids to document overall treatment performance. Soluble BOD and COD analyses were performed on the filtrate of samples filtered through glass fiber filter paper. All BOD analyses were inhibited to prevent nitrification. Analytical methods were done in accordance with the 15th edition of Standard Methods (7).

The net solids production (kg TSS/kg BOD removed) was determined by measuring the mass of solids exiting in the backwash water and in the system effluent. The quantity of backwash water siphoned from a BAF cell during each backwash cycle was relatively constant. Backwash water was collected in a tank whose volume had been calibrated as a function of liquid depth. A microprocessor provided a counter and LED readout of the number of backwash cycles. A backwash sample of approximately 1 liter was pumped from a cell at the beginning of each backwash cycle siphon. A signal to the sample pump was controlled by the microprocessor. At the time of sampling, the backwash water was well mixed due to the water flush through the cell.

Two methods were used to determine the amount of oxygen consumed. The first and more direct method involved measuring the air sparge rate and sampling and analyzing the oxygen content of the off gas escaping through the liquid at the top of the BAF cell. The air supply rate was measured by the use of an annubar and manometer located on each of the air supply lines to each BAF cell. Temperature and pressure measurements were used to calculate the air rate at standard conditions. A 0.61 m (2 ft) x 0.91 m (3 ft) fiberglass tank was immersed in the liquid with the open end down to collect the gas. A tube at the center of the gas collection device directed the gas to a gas sampling tube. The oxygen and carbon dioxide content of the gas samples were measured in the laboratory using an Orsat apparatus and procedure (8). Gas samples were taken at nine different locations uniformily located across the top of each BAF cell. During the off gas sampling period, the feed flow rate was maintained constant and influent and effluent grab samples were obtained for COD analysis. The second method was to conduct a COD balance on the system based on the COD of the influent, effluent, and backwash water. While less accurate, this method allowed a daily oxygen utilization rate calculation for a complete 24 hour period and the COD data were available daily. In the off gas method, oxygen utilization rates were only observed over a 1-2 hour test period.

BAF TREATMENT PERFORMANCE

BOD AND TSS REMOVAL

The primary effluent wastewater characteristics are divided into four periods in Table 2 according to the influent temperature conditions. From June through October, the influent temperature was relatively warm ranging from 18 to 23°C. During November, the wastewater temperature steadily declined from 17 to 12°C. From December to early March, the wastewater temperature remained relatively cold and then gradually increased from 12 to 17°C from April through May. The primary effluent was 20 to 30 percent weaker than normal during this study phase as a result of unusually high precipitation. As shown, the soluble BOD concentration was only 20-25 percent of the total BOD concentration. This shows that a large portion of the BOD removed in the BAF system was due to solids removal by filtration with the media bed.

TABLE 2. PRIMARY EFFLUENT AVERAGE WASTEWATER CHARACTERISTICS

DATE	Temp °C	TBOD (mg/l)	SBOD (mg/l)	TSS (mg/l)
June-October 1983	21 (18-23)	91	20	123
November 1983	15 (12-17)	89	20	108
December-March 1984	12 (10-13)	100	24	113
April-May 1984	15 (12-17)	92	21	117

West cell average organic loading rates, temperatures and effluent BOD and TSS concentrations are summarized in Table 3 for the initial operating period prior to changing the media in March 1984. During the warmer operating period from July to September, the average weekly organic loading ranged from 3.5 to 6.0 kg BOD/m^3-day (220 to 370 lb/1000 cu ft-day) and the effluent BOD and TSS concentrations averaged 13.0 and 14.3 mg/l, respectively, for that period. During the latter part of September, when the digester cleaning operation resulted in very high influent solids concentrations and higher organic loadings, the BAF effluent TSS concentration increased but the effluent BOD concentration was not significantly affected. During the high backwash frequency in October due to an apparent influent fiber problem, the effluent TSS and BOD concentrations decreased, even though the organic loading was higher.

TABLE 3. WEST CELL AVERAGE PERFORMANCE

PERIOD	AVERAGE ORGANIC LOADING kg BOD/m^3-day	AVERAGE TEMP °C	EFFLUENT CONCENTRATIONS (mg/l)	
			BOD	TSS
July - September 1983	4.7	21.5	13.0	14.3
September-Digester Cleaning (2 weeks)	5.0	21.3	11.6	16.5
October	4.9	19.6	10.0	8.8
November-December	4.3	12.0	12.6	10.8
January-February 1984	4.4	11.3	22.7	20.4

During the colder operating months, both organic loading and wastewater temperature were changing on a daily and weekly basis. The data shows that the system effluent BOD and TSS concentrations increased significantly during the colder temperature operation in January and February.

As Table 4 shows, the lower loaded East cell produced effluent BOD and TSS concentrations close to 10 mg/l, with organic loadings in the 3.0-3.5 kg BOD/m^3-day (180-220 lb/1000 cu ft-day) range. As in the West cell operation, a higher effluent TSS concentration occurred during the digester cleaning operation. During the high backwash frequency operation associated with the appearance of fibers, the East cell effluent BOD and TSS concentrations were lower even though the organic loading was not decreased. As observed for the West cell effluent the effluent BOD and TSS concentrations increased during the colder temperature operation in January and February.

TABLE 4. EAST CELL AVERAGE PERFORMANCE

PERIOD	AVERAGE ORGANIC LOADING kg BOD/m^3-day	AVERAGE TEMP °C	EFFLUENT CONCENTRATIONS (mg/l)	
			BOD	TSS
July - September 1983	3.2	20.6	11.8	9.6
September Digester Cleaning	3.5	21.1	9.6	12.9
October (High Backwash)	3.2	19.6	7.2	6.4
November-December	3.5	13.2	11.3	9.1
January-February 1984	4.1	11.4	17.8	16.1

Since wastewater characteristics, strength, and temperature could all vary simultaneously, it was not possible to separate performance variables to precisely determine relationships describing treatment performance. However, organic loading and temperature are parameters that affect performance. The effect of these parameters on effluent BOD concentration is shown in Figure 3. The data points are separated into two categories: wastewater temperatures greater than 18°C and wastewater temperatures less than 15°C. The October and November performance data are not included in this analysis because of the changing wastewater temperatures and the presence of fibers in the influent. The April to May data are also not included in Figure 3 because a different media size was used during that operating period. There was less scatter in the data at temperatures greater than 18°C, and a linear regression yielded the straight line shown with a correlation coefficient of 0.89. A similar analysis for the lower temperature data yielded a correlation coefficient of 0.80. The data show that the average weekly effluent BOD concentration is higher and more sensitive to organic loading at the lower temperature range. At lower organic loadings the effluent BOD concentration is not as significantly affected by the wastewater temperature ranging from 10 to 23°C. At an organic loading of 5.0 kg BOD/m^3-day (310 lb/1000 cu ft-day) the effluent BOD concentration increased by about 40 percent when the temperature was decreased to the lower operating range. Effluent TSS concentrations followed the same trend as effluent BOD concentrations throughout the study.

As indicated in Figure 4, lower effluent BOD concentrations were achieved in October and November over a wide range of organic loadings than during other operating periods. Performance was also relatively insensitive to wastewater temperature which dropped from 18 to 12°C during this period. Previous performance data obtained for temperatures greater than 18°C are included in the shaded area on the figure. The lower effluent BOD and TSS concentrations during this period are due to improved filtration performance. A more rapid headloss buildup and higher backwash frequency was also observed. These results appear to be related to fibers in the influent wastewater to the BAF cells. Visual examination at the top of the media bed showed that the top few inches of media contained a mat like structure consisting of an agglomeration of media, small fibers, filtered solids and attached biological solids. This material provided excellent filtration at the top of the bed at the expense of a very rapid headloss build-up.

These performance results indicate the importance of wastewater temperature and wastewater characteristics on treatment system efficiency. BOD removal efficiency can not be assumed to be only a function of organic loading, which has been used previously as the basic performance parameter. For the BAF system, filtration is an important particulate BOD removal mechanism along with mass transfer and biological oxidation for removal of soluble degradable organics in the wastewater. Thus, the effluent BOD that may be achieved at a given organic loading will depend on the proportion of particulate BOD in the influent and the filterability of these solids. As illustrated by the fibers occurrence, the backwash requirements are also affected by influent solids characteristics.

Soluble COD removal profiles obtained from grab samples at taps located along the media depth, indicated that most of the soluble organic removal

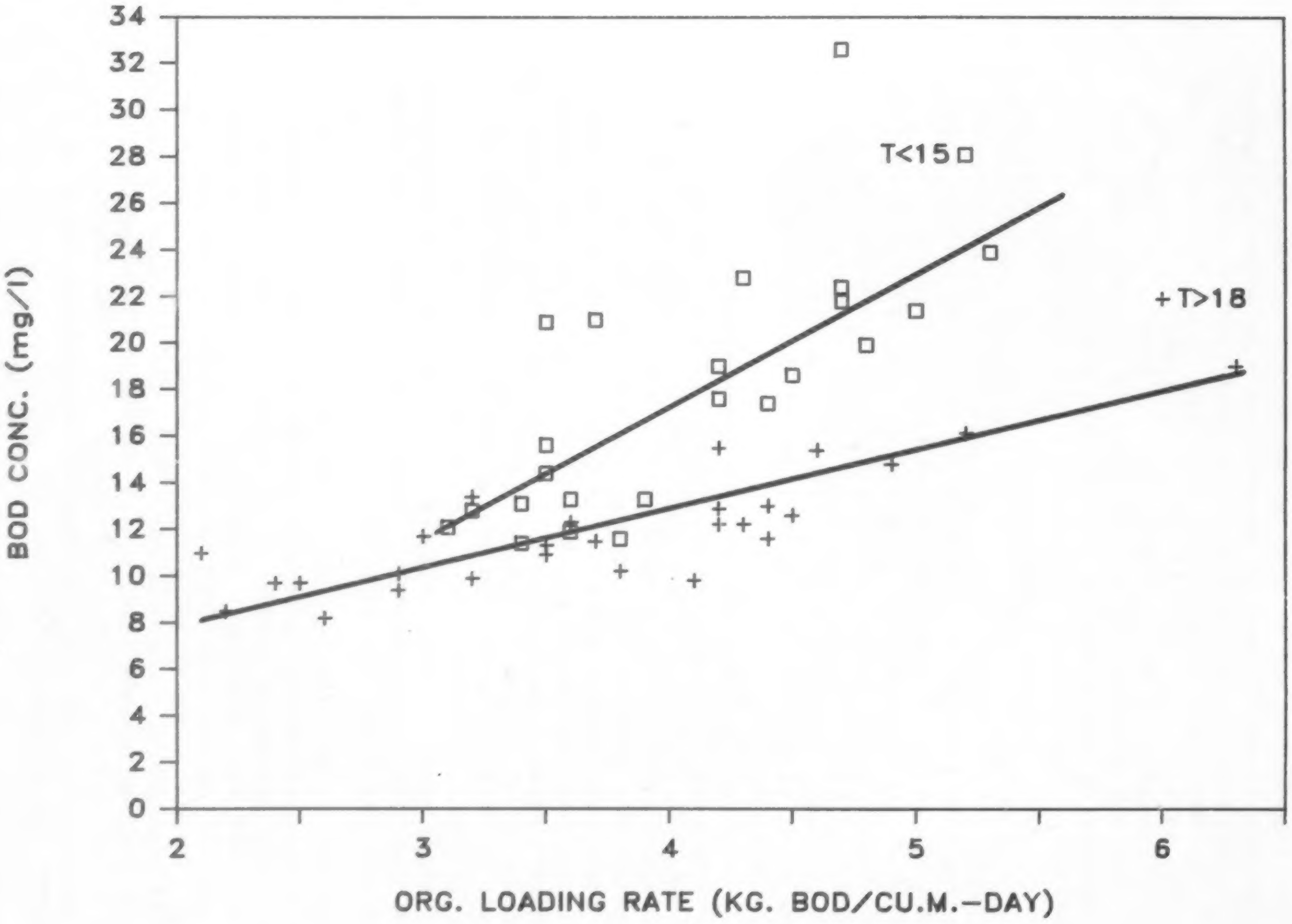

Figure 3. Effluent total BOD versus organic loading rate-weekly averages.

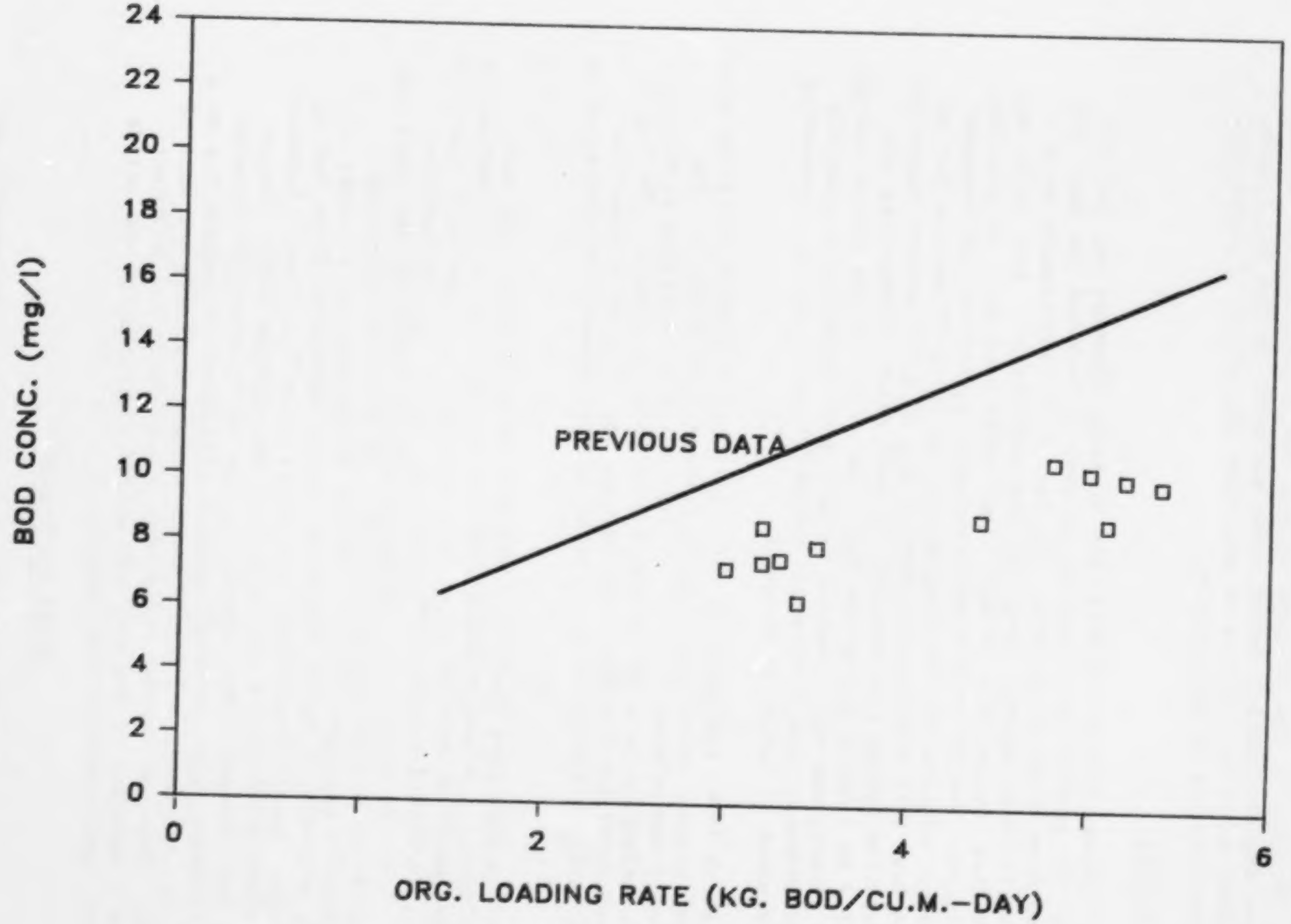

Figure 4. Influent fibers affect effluent BOD.

occurred in the first 0.9 to 1.2 m (3 to 4 ft) of media. This suggests that the same effluent quality may have been achieved if the organic loading was increased by an increase in the influent soluble BOD concentration. Unless very conservative organic loading design value are used for the BAF System, pilot plant studies would be required to determine the effect of different wastewater characteristics on performance.

BACKWASH REQUIREMENTS

As Figure 5 shows, before and after the digester cleaning and influent fiber occurence, the percent of product water used to backwash the West cell generally ranged from 10 to 15 percent. The East cell requirements were generally in the 15 to 20 percent range for the same period. Both cells were backwashed with five cycles on a 24 hour timed basis each day. In addition, one or two additional two cycle backwashes occurred in October and November with product water requirements reaching as high as 20 to 30 percent for the West cell. The East cell requirements were in the 30 to 45 percent range for one week of operation during this period. In such cases, backwashing was occurring every 2 to 4 hours during the day. The source of the fibers was not determined. A laundry and small leather tannery were suspected as possible sources. After November, the problem did not occur again and the backwashing requirements returned to normal in December.

In early March 1984, the media in both cells was changed to a larger size and the backwash product water requirements dropped significantly. The backwash frequency was reduced to once per day, which is desirable to minimize hydraulic load variations to a BAF system due to recycle streams. The higher percent backwash water for the East cell in late May 1984 was a result of decreasing the influent hydraulic application rate to promote nitrification in the cell.

These results show that there is a tradeoff between media size and backwash requirements. A larger media size may be desirable in the BAF system to minimize the amount of product water recycled through the primary clarifier and BAF cells. Increased backwash frequency can impact system economics several ways. The size of primary clarifiers and the number of BAF cells are affected by increased system hydraulic loads. In addition, increased backwashing requires that each BAF cell be out of service for a longer time each day, thus requiring a larger system to handle wastewater flows during backwash periods. Energy requirments also increase with increased backwash requirements since backwash air and water must be supplied over a longer duration each day. However, when the media size is increased to minimize backwash requirements, the system's treatment efficiency is reduced due to a lower filtration efficiency and less biofilm surface available per unit of reactor volume. Thus, an optimal media size likely exist for different wastewaters as a function of the influent solids concentration and solids characteristics, and treatment level needed.

MEDIA COMPARISON

As Figure 6 shows, to meet desired treatment levels, the organic and hydraulic loading rates were decreased to both BAF cells after changing the

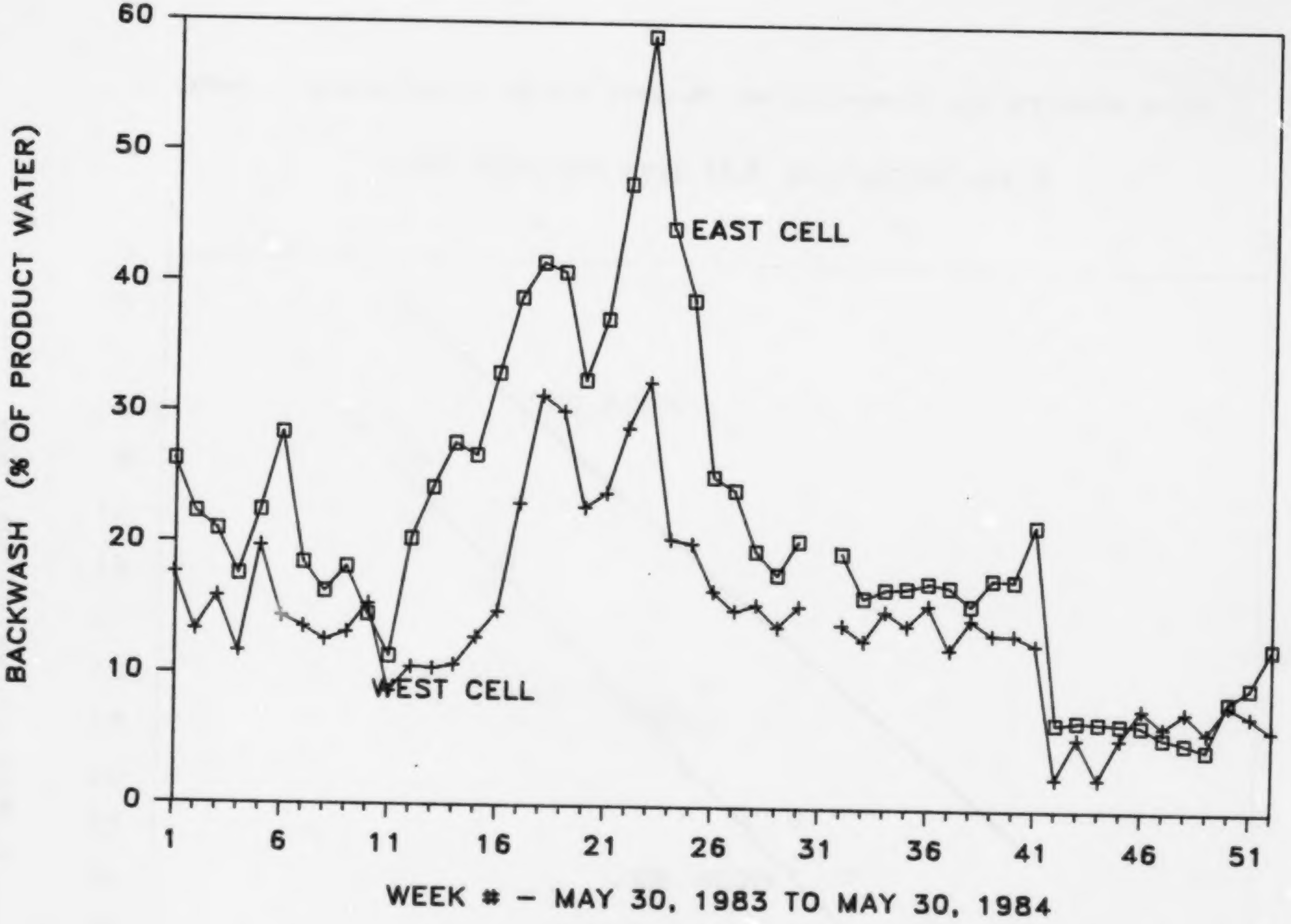

Figure 5. Percent of product water used for backwash - weekly average.

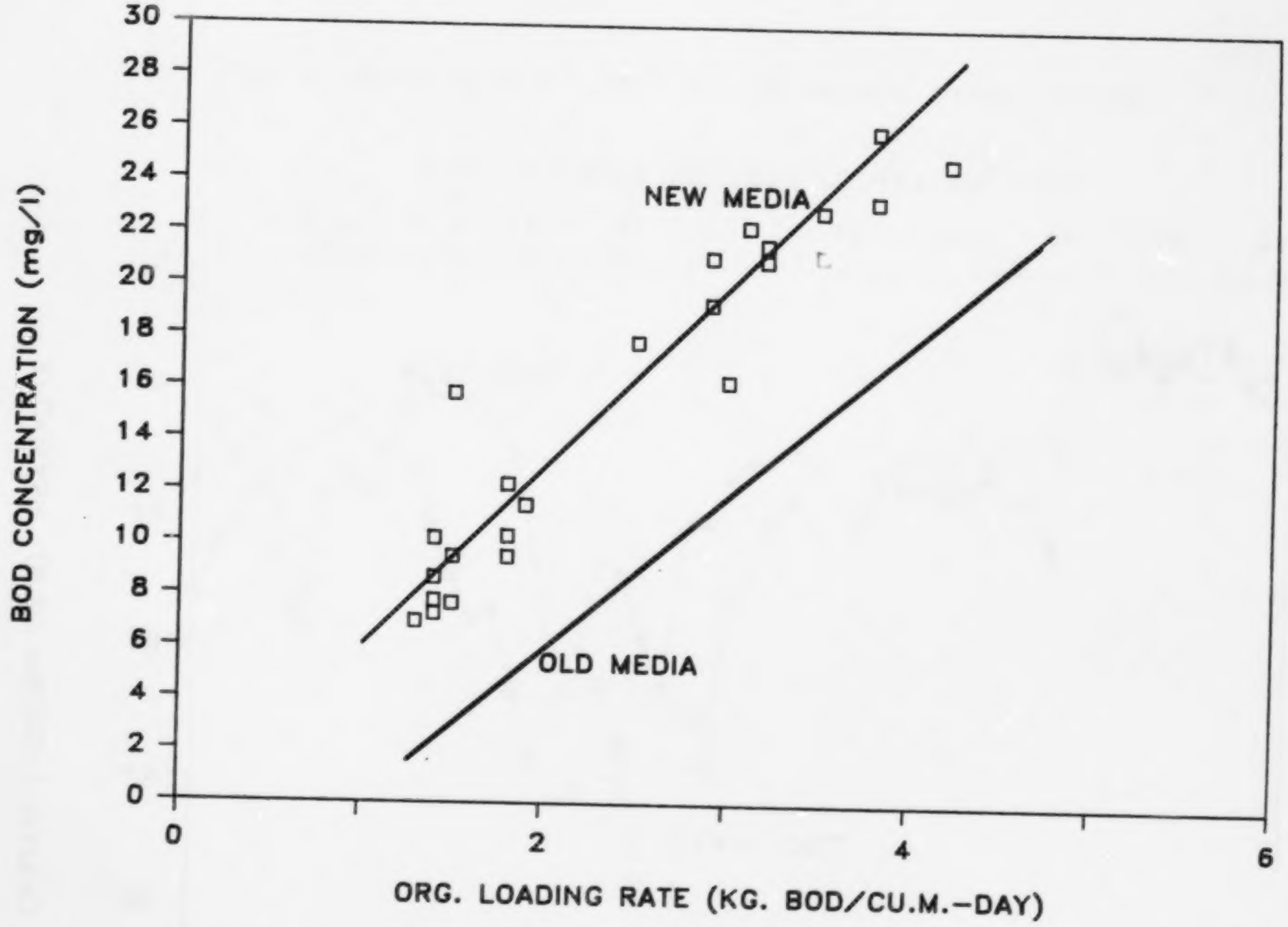

Figure 6. Comparison of organic loadings and performance for different media.

media. The West cell was operated at about the same organic loading rate used previously for the East cell and the East cell organic loading rate was reduced to 1.5 to 2.0 kg BOD/m^3-day (90-125 lb BOD/1000 cu ft-day) to achieve a treatment goal of both BOD removal and nitrification. Table 5 compares the BAF performance for the two different media sizes for operating periods with similar wastewater temperatures and organic loadings. Both systems were operated under constant flow conditions during these periods. Even though the new media was operated under a slightly lower organic loading rate than used for the earlier media the effluent TSS and BOD concentrations were significantly greater. However, the backwash water requirement was significantly reduced with the new media.

An overall comparison between media BOD removal performance is further shown in Figure 7. A linear regression of effluent BOD versus organic loading for the new media yielded a straight line with a correlation coefficient of 0.85. This is compared to the straight line obtained for data collected on the initial media. The sensitivity of effluent BOD concentration to organic loading was similar in both cases. The figure clearly shows the reduced treatment capacity for the larger sized media. An effluent BOD concentration of 20 mg/l may be achieved at an organic loading of 3.0 Kg BOD/m^3-day (190 lb BOD/1000 cu ft-day) for the new media versus about 4.5 kg BOD/m^3·day (280 lb BOD/1000 cu ft-day) for the old media. The lower treatment efficiency for the new media is attributed to poorer filtration efficiency and lower biofilm surface area per unit volume.

TABLE 5. BAF MEDIA EVALUATION

Date	INITIAL MEDIA 11/28 to 1/19		NEW MEDIA 3/26 to 4/30	
Wastewater Temperature (°C)	12.4 (12.0 - 12.6)		12.7 (12.0 - 13.5)	
Organic Loading (kg BOD/m^3-day)	3.8 (3.4 - 4.4)		3.4 (3.1 - 3.8)	
Average Performance	Influent	Effluent	Influent	Effluent
TBOD (mg/l)	88	13.4	94	21.7
TSS (mg/l)	107	10.8	123	22.5
Percent Backwash	14.8		6.8	

OXYGEN UTILIZATION AND SLUDGE PRODUCTION

Oxygen consumption per unit of BOD removed was determined from off-gas tests and from the COD balance calculations. The average values and corresponding ranges of values determined with each method are summarized in Table 6. The oxygen consumption per unit of BOD removal was similar for both cells even though the organic loadings were different. This may be related to

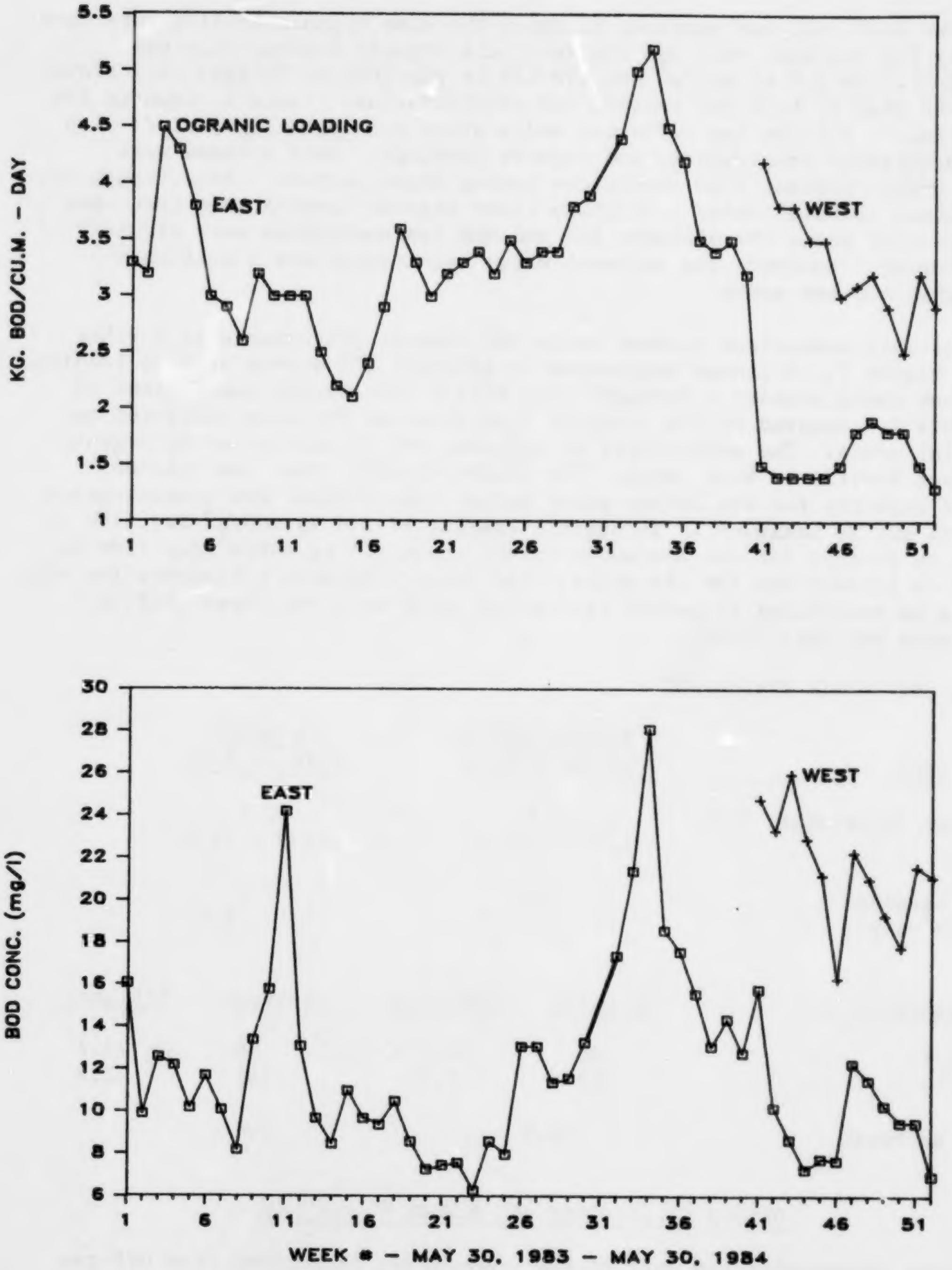

Figure 7. Effluent BOD versus organic loading rate for new media.

the fact that the solids filtered out in both systems may have remained in each cell about the same amount of time, since both cells were backwashed at least every 24 hours. The oxygen consumption ratios are low compared to conventional or highly loaded activated sludge treatment systems. Since much of the influent BOD was in the form of particulate matter, this suggests that the solids filtered were not highly degraded. Filtered solids may be retained in the BAF for a matter of hours before backwashing compared to days for activated sludge systems. Thus, less solids degradation may be expected. The portion of soluble BOD in the influent may also affect the oxygen consumption. As the soluble fraction of the influent BOD increases, the oxygen consumption ratio can be expected to increase since a greater portion of the influent BOD should be oxidized.

TABLE 6. OXYGEN CONSUMPTION IN BAF CELLS

	Oxygen Consumption (kg O_2/kg BOD Removed)	
	East Cell	West Cell
Off Gas Tests	0.55 (0.43 - 0.69)	0.51 (0.42 - 0.80)
COD Balance Method	0.66 (0.40 - 0.96)	0.63 (0.44 - 0.82)

The solids production per unit of BOD removed was similar for both cells as shown in Figure 8. These observations agree with the previously noted similar oxygen consumption values for each cell. The solids production values were higher during the digester cleaning operation that resulted in substantially increased influent solids concentration. The lowest value occurred for the week of January 16. During that week, the influent soluble BOD fraction was higher than previous weeks. This further supports the hypothesis that soluble BOD is more readily degraded in the BAF cell than particulate BOD.

Based on the observations in Figure 9, the solids production yield factor was plotted against the ratio of TSS to BOD in the BAF influent. As Figure 9 shows, a relationship was apparent with a correlation coefficient of 0.89. Figures 8 and 9 indicate that the solids production and oxygen consumption per unit of BOD removed are a function of the influent wastewater characteristics.

SUMMARY AND CONCLUSIONS

This paper summarizes a one year evaluation of a full scale BAF system treating a municipal wastewater plant primary effluent. The operation was completely automated with a microprocessor controller, and operator requirements were limited to equipment maintenance and sampling. Treatment

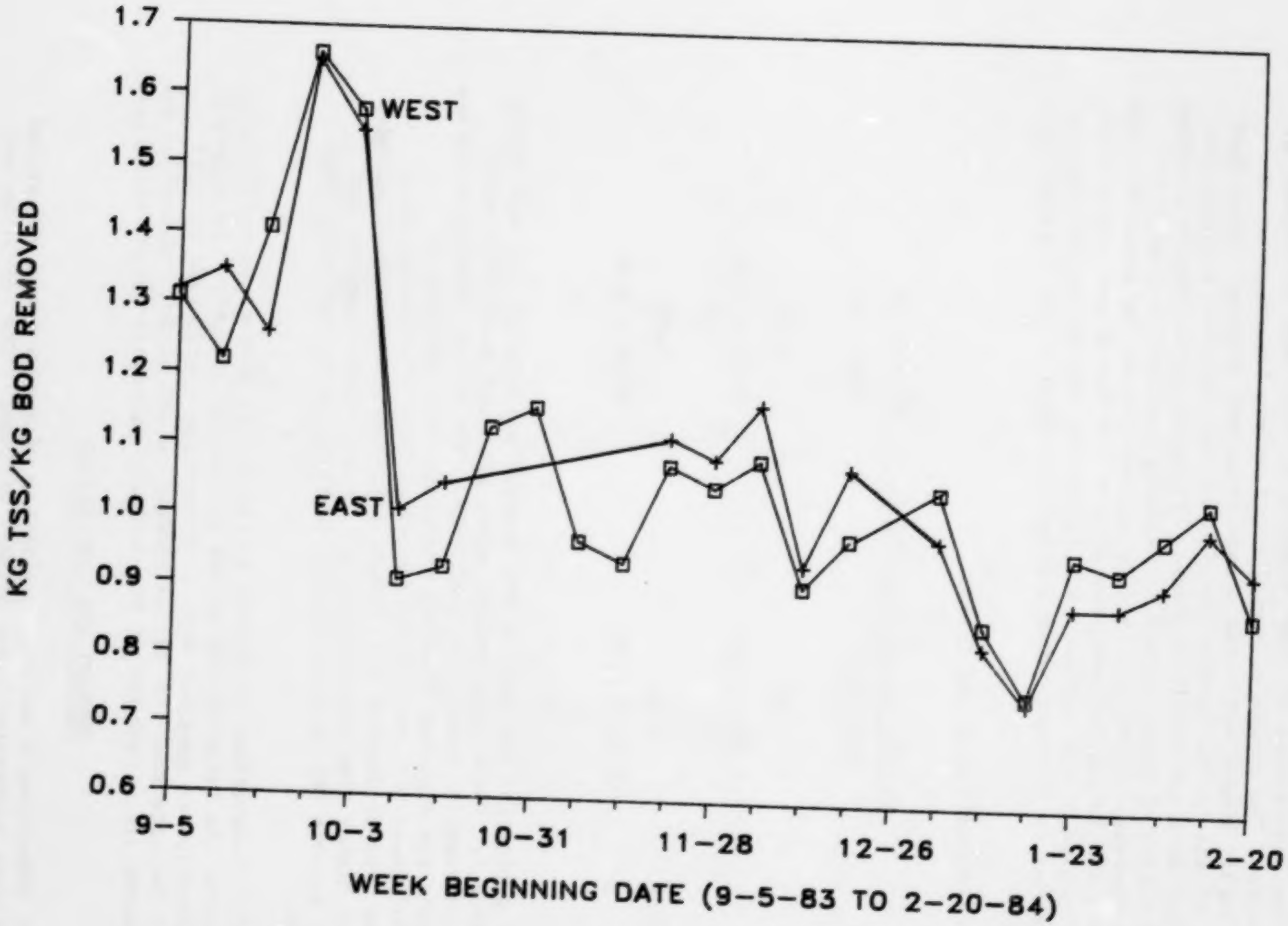

Figure 8. Solids production rate for East and West BAF cells.

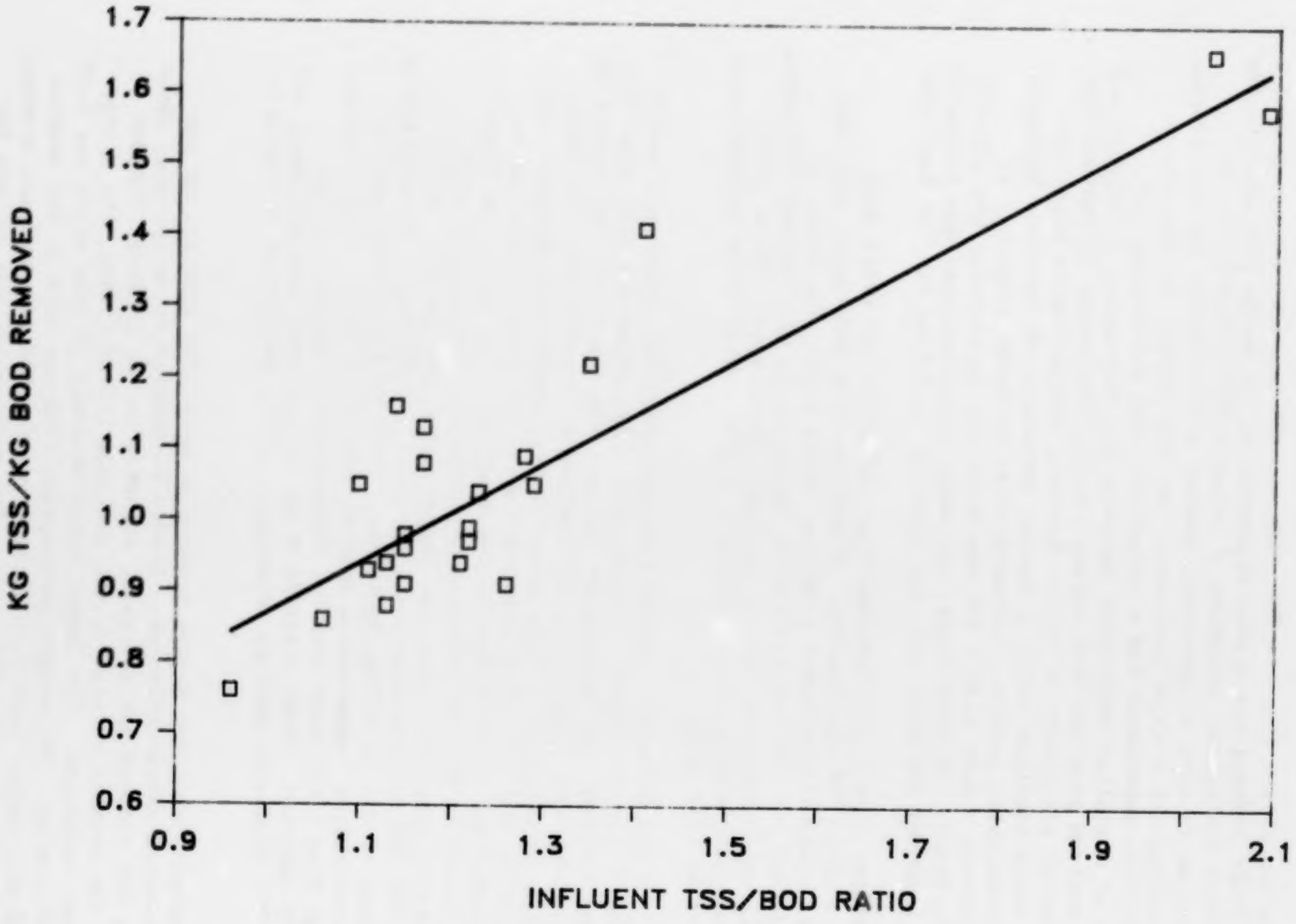

Figure 9. Solids production rate affected by influent TSS and BOD ratio.

performance parameters evaluated were BOD removal, TSS removal, oxygen consumption, sludge production and backwash frequency.

The evaluation showed that the BAF, BOD and TSS removal efficiencies as a function of organic loading are highly dependent on the media size used in the BAF cell. Besides biological oxidation of soluble BOD diffusing to the biofilm, filtration is also an important removal mechanism for the BAF system. Filtration accounted for a major portion of the BOD removal in this study because the influent wastewater had a relatively high particulate BOD fraction. Use of a smaller media size improves the filtration efficiency and provides more surface area per unit volume for biofilm growth to improve the soluble BOD removal efficiency as well. However, smaller media sizes can create a more rapid headloss build up during operation and increase backwash frequency to undesirable levels. A once-per-day backwash frequency is desirable to provide a more stable BAF operation and to minimize the effects of high recycle flows. In this study, the media effective size was increased from 2.8 to 4.4 mm for the West cell and from 2.8 to 3.4 mm for the East cell.

The wastewater characteristics may also influence the media size selection for a given BAF application. A more soluble wastewater with less influent solids would likely favor a smaller media size. The level of influent solids and the characteristics of those solids will affect filtration and BOD removal performance as well as backwash requirements for a given media. Headloss problems occured when the influent contained an unusual level of small fibers in this study.

Expected BOD and suspended solids removals for a BAF system may not be predicted from the design organic loading rate only. Removals will also be affected by the influent particulate and soluble BOD fractions, the nature of the influent solids, the media size, and wastewater temperature. At lower loadings, system performance is less sensitive to temperature changes. BAF system effluent BOD and TSS concentrations increased significantly as the loadings increased for temperatures in the range of 12 to 15°C. At higher temperatures (above 18°C), the performance decreased less rapidly with increasing organic loadings. With the larger media used in this study, effluent BOD and TSS concentrations of less than 25 mg/l could be achieved at organic loadings of 3.5 to 4.0 kg BOD/m^3-day (220 to 250 lb BOD/1000 cu ft-day) with wastewater temperatures ranging from 12 to 17°C. This loading range is about five times that used for conventional activated sludge treatment. In addition, final clarifiers and sludge recycling equipment are eliminated. The land area savings advantages of the BAF system are readily apparent.

Oxygen requirements and sludge production were also affected by the influent wastewater characteristics. Sludge production rates averaged about 1.1 kg TSS/kg BOD removed but increased relative to the influent TSS/BOD ratio. When the soluble portion of the influent BOD increased, the sludge production rate decreased. The lowest value observed in this study was 0.75 kg TSS/kg BOD removed. The oxygen consumption rate per unit of BOD removed varied over a wide range (0.40 to 0.96 kg Oxygen/kg BOD removed) and averaged from 0.62 to 0.66 for the two BAF cells. The particulate and soluble BOD fractions of the influent wastewater likely affected these values.

The results of this study provide useful information on factors that affect the BAF system performance and also provide a range of design parameters for municipal wastewater treatment applications. Conservative values for design should be used unless pilot plant studies are performed to evaluate the treatment performance and backwash requirements for a specific wastewater and media selection.

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FINE PORE AERATION PRACTICE

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FINE PORE AERATION PRACTICE

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ABSTRACT

This paper describes the current state-of-the-art of fine pore aeration of municipal wastewaters. It represents a condensation of an interim guidelines report that will be published by United States Environmental Protection Agency (U.S. EPA) in the Fall, 1985. The material presented herein is the result of effort by the ASCE Subcommittee on Oxygen Transfer.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agencies peer and administrative review policies and approved for presentation and publication.

INTRODUCTION

Aerobic biological processes continue to be one of the more popular methods employed to treat municipal and industrial wastewaters. The supply of oxygen to the biomass in activated sludge and aerated lagoons represents the single largest energy consumer in the treatment plant. Recent studies indicate that from 50 to 90 percent of the net power demand for a treatment plant lies within the aeration system (1). A rather general survey of data available in 1982 on municipal and industrial wastewater treatment installations suggests that in the North American Continent there are approximately 1.75 million installed horsepower of aeration equipment in place at an installed value of 0.6 to 0.8 billion dollars (2). Operating costs for these systems may be expected to be about 0.6 billion dollars per year.

Originally, oxygen was diffused into wastewater through perforated pipes located at the bottom of the aeration tank. The development of the porous plate for aeration was considered an important advance in the diffused air process because of the high transfer efficiency of this fine pore device(3). Porous diffuser plates were used as early as 1916 and became the most popular method of aeration in the 1930s and 1940s (4,5). It was clear shortly after the development of porous diffusers that clogging could be a problem. Early work on clogging led to the use of coarser media (6) and eventually to large orifice devices (7). Use of mechanical aeration devices was another answer to the clogging problem although these devices were normally applied to small treatment facilities and industrial waste applications (7).

The energy crisis of the early 1970s brought new awareness within the sanitary engineering community relative to the efficiency of oxygen transfer systems. Although there is some controversy over which aeration device provides the most favorable performance, the fine pore diffusion of air has gained renewed popularity as a very competitive system. Yet considerable concern has been registered regarding the performance and maintenance of fine pore systems owing to their susceptibility to clogging. Diffuser clogging, if severe, may lead to deterioration of aeration efficiency resulting in an escalation of power costs. Furthermore, troublesome maintenance of diffusers may consume considerable amounts of operator time and plant operations money.

The term "fine bubble" aeration is elusive and difficult to define in specific terms. For purposes of this report, the term fine pore diffusers is used instead of fine bubble to more nearly reflect the characteristic of the diffusers themselves. Fine pore diffusers are defined herein as porous air diffusing devices that produce an initial component of surface tension equal to or greater than 5 cm (2 in) water gauge.

The objective of this report is to provide current information on the performance, operation, maintenance, and retrofitting of fine pore aeration in municipal wastewater treatment systems.

TYPES OF FINE PORE DIFFUSERS

FINE PORE MEDIA

There exists in the marketplace today a number of porous materials capable of serving as effective aeration devices. In general, a wide range of products which were initially developed to filter air or liquid have also been found to act as a satisfactory air diffusion device. Because of cost and specific characteristics, only a relatively few types of materials are actually being used in the wastewater treatment field.

Ceramic Materials

The oldest and still the most common type of porous material on the market is the so-called ceramic type. As a general description, it consists of crystalline or irregular shaped particles bonded together into various shapes to produce a network of interconnecting passageways through which the air flows. As the air emerges from the surface pores, pore size, surface tension, and flow rate interact to produce the characteristic bubble size (8). There are a number of types of ceramic diffusers including glass and resin bonded silica and alumina. The two most popular types are the glass fused silica and alumina.

The silica material is produced from naturally occurring sand particles. After screening to obtain the desired uniform particle size, an amorphous glass binder is added. The aggregate and binder mix is then pressed in a mold to produce the desired shape. After pressing, the material is fired at approximately 980°C (1800°F). At this temperature, the binder material encapsulates the sand particles. When the mix is cooled, a glass bond is formed by the binder material at the contact points between the individual particles.

The fused alumina material is made from aluminum oxide. The actual grains are produced by melting bauxite ore at approximately 1120°C (2050°F) to form large pigs. The pigs are then crushed and the resulting particles screened to select the desired size. For the alumina, a more elaborate binder resembling procelain is used. After pressing, the grit and binder mix is then fired at 1425°C (2600°F) which upon cooling creates the glass bond at the contact points. The final product is typically 80-90% aluminum oxide.

There are a few minor differences between the two types of material. Because of the crushing process, the alumina grains are more angular and jagged in shape compared to the silica. Because it is a mined material with a limited particle size range, the pore size that can be produced with silica is limited by naturally occurring grain sizes. This prevents its use in applications where a large pore opening is desired.

Today, the majority of ceramic diffusers being marketed are manufactured from aluminum oxide. The alumina material is harder and possibly somewhat stronger than the silica, but this alone may not be the reason for its widespread use.

Plastic Materials

A more recent development in the fine pore field is the use of porous plastic materials. Similar to the ceramics, a material is created consisting of a number of interconnecting channels through which the air can pass. Advantages of the plastic material over aluminum oxide are its weight (the light weight makes it especially suited to lift out applications), cost, durability, and depending upon the actual material, its resistance to breakage. Disadvantages include strength, creep resistance, and variable dynamic wet pressure with time due to a change in contact angle.

Porous plastics are made from a number of thermoplastic polymers including polyethylene, polypropylene, polyvinylidene fluoride, ethylene-vinyl acetate, styrene acrylonitrile, and polytetrafluoroethylene (14).

Probably the most common type of plastic materials in use are high density polyethylene (PE) and styrene acrylonitrile (SAN). PE is used because it is relatively easy to process compared to other thermoplastics. Shrinkage is low, a uniform quality product can be obtained, and small pore sizes can be produced. The actual material is manufactured by a proprietary process, and thus little information is available.

The major advantages of the PE media is that it is lightweight (approximately 560 kg/m^3 (35 lbs/ft^3), essentially inert, and will not break, even when frozen. Potential disadvantages are strength, creep, and variable contact angles. Furthermore, it is a relatively new product (at least as an air diffusion device) and all of the long-term effects may not be known.

The second most common type of thermoplastic material is styrene acrylonitrile (SAN) copolymer. The raw material is a mixture of four different molecules. Physically the media is made up of very small resin spheres which are fused together under pressure. The SAN media has a density only slightly greater than PE. The presence of the styrene, however, makes the material brittle and the media can break if dropped, even at room temperature. A major advantage of the SAN material is that it has been in use for approximately 15 years without known deleterious effects.

Flexible Membranes

Flexible type diffusers have been in use for approximately 40 years. They initially were referred to as "sock diffusers" and made from materials such as plastic or synthetic fabric cord or woven cloth. As with plastic tube diffusers, a metallic or plastic core material is necessary for structural support. Although sock diffusers were capable of achieving relatively high oxygen transfer rates, fouling problems were often severe. Today, there is essentially no market for the early sock design.

Within the last several years, a new type of flexible diffuser has been introduced. It consists of a thin flexible sheath made from soft plastic or rubber material. Air passages are created by punching minute slots in the sheath material. When no air is being applied, a check valve at the inlet prevents backflow into the distribution piping. When the air is turned on, the sheath expands. Each slot acts as a variable aperture opening, the higher the flow rate the greater the opening. The sheath material is supported by a plastic tubular frame.

PHYSICAL CHARACTERISTICS OF MEDIA

There are a number of physical properties of the media that are important to the design engineer. These include permeability, porosity, pore size, uniformity, dynamic wet pressure, strength, chemical stability, heat resistance, and density.

Permeability

"Permeability is defined as the volume of air in cubic feet per minute which is passed through one square foot of diffuser, tested dry, at 5 cm (2 in) water differential pressure, under standard conditions of temperature and humidity. This permeability rating has been the accepted standard of comparisons for some 30 years, for want of a better practical test. However, it does not give a true basis for comparisons of performance, because the same permeability rating could be obtained from a diffuser with a few relatively large pores or a multitude of fine pores. Also, two diffusers with exactly the same pore structure would have different ratings if of different thickness. It appears that a supplementary specification is necessary to give a practical measure of the number and size of the pores in a diffuser of given permeability. This is currently being studied . . ." (5).

The quotation above from the 1952 Air Diffusion in Sewage Works Manual still reflects the status of permeability measurements in 1985, although the study of supplementary methods has greatly intensified and should lead to a more rational procedure for specification of porous diffusers.

As best can be determined, the ceramic industry has not "standardized" this test procedure. The early specifications were developed for 30.5 x 30.5 cm (12 in x 12 in) plates of 2.5 cm (1 in) and 3.8 cm (1.5 in) thickness. Today, specifications are needed for products of various shapes, densities, and wall thicknesses, often of ill-defined effective area. Attempts have been made to apply the principles of the test through a parameter known as specific permeability (8). In its determination, an airflow is measured through a diffuser mounted on a fixture similar to the one used in service at a pressure differential of 5 cm (2 in) water gauge. From this measurement and the geometry of the diffusers, estimates are then made as to what the airflow would have been had the dimensions of the test diffuser been 30.5 cm x 30.5 cm x 2.5 cm(12 in x 12 in x 1 in).

The specific permeability procedure has served to improve the utility of this test but does not overcome the remaining deficiencies which include:

- Clamping and sealing details are not well enough defined to provide acceptable precision.
- Correction factors to account for pressure, temperature, and humidity have not been developed.
- The test indirectly measures a characteristic of real interest which is only slightly more difficult to measure directly and with greater accuracy (Dynamic Wet Pressure on Bubble Release Vacuum (11)).

The major redeeming feature of the existing test procedure is its simplicity. Its use should probably be retained, at least temporarily, until more suitable standardized procedures are available.

Porosity and Pore Size

Porosity is defined as the ratio of pore volume to total volume of the material. Porosity is relatively difficult to measure and does not have well-defined relationships with the functional characteristics of porous diffusers (other than density); consequently, it is rarely specificed by the engineer or owner. A porous media will have pores which can be classified as open, closed, or open only at one end. Since the porosity includes the volume of all pores, even though only the open pores will transmit gas, the porosity value can be misleading.

For ceramic diffusers, the porosity is usually in the 30 to 40% range. For a specific manufacturer, the porosity is about the same regardless of the grade of material. Different grades are simply made by varying the combination of size and number of pores. As a result, porosity and permeability are not synonymous or directly related. Yet, pore size or effective pore sizes is a characteristic of considerable significance since it gives some indication of wet pressure and uniformity.

Uniformity

Uniformity of individual diffusers and the entire aeration system is of extreme importance if high efficiency is to be maintained. On an individual basis, the diffuser must be capable of obtaining uniform air distribution across the entire surface of the media. If dead spots exist, chemical or biological foulants may form and eventually lead to premature fouling of the diffuser. Also, if small areas of extremely high flow are present, larger bubbles may form. The diffuser will "coarse bubble" and oxygen transfer efficiency will be reduced.

Like permeability, a porous diffuser specification should include a requirement for testing to assure that the media will distribute air uniformly. A method which will quantify this uniformity actually measures the rate of air release from different areas of the diffuser (10). With the diffuser submerged in 5 to 20 cm (2 to 8 in) of water and at an airflow rate of approximately 36 $m^3/hr/m^2$ (2 $scfm/ft^2$), the rate of air release is determined by measuring the displacement of water from an inverted cylinder.

Based on air volume, time, and the area of the collection cylinder, a flux rate is determined. A comparison of the flux from various points will give a true indication of the uniformity. Although procedures have been presented (10), no guidelines have yet been developed in regard to the variations between points which could exist before the diffuser would be rejected as nonuniform. Development of such guidelines should be undertaken.

In addition to individual diffusers, flow characteristics throughout the system must also be uniform to assure equal airflow rates throughout the aeration tank. System uniformity should not be a problem if the diffusers pass the permeability requirements described in the preceding section, provided suitable individual control devices are provided.

Wet Pressure Loss (Dynamic Wet Pressure)

Wet pressure loss can also be an important consideration in evaluating or selecting a porous media. As a general rule, the lower the permeability, the smaller the bubble size, and the higher the headloss. While a small bubble may increase the oxygen transfer, the additional power required to overcome the headloss may negate any potential savings.

The porous media currently in use today has a wet headloss over the typical operating flow range of 10 to 25 cm (4 to 10 in) of water. The specific value depends on the flow rate, type of material, thickness, and surface properties. For the ceramic and porous plastic materials, the headloss versus flow curve is linear over the typical operating range and the slope is relatively flat. A fourfold increase in airflow 1 to 3 m^3/hr (0.5 to 2.0 scfm) per unit for some diffusion elements will result in only a 2 to 5 cm (1 to 2 in) increase in headloss across the media itself. For the flexible material, the small holes act like an orifice. As a result, the headloss versus flow curve is steeper (11). Over the typical operating range of 3 to 10 m^3/hr. (2 to 6 scfm) per unit, the headloss may increase from 13 to 38 cm (5 to 15 in).

For the ceramic and plastic material, the majority of the wet headloss is associated with the pressure required to form bubbles against the force of surface tension. Only a small fraction of the total headloss is required to overcome the frictional resistance (10). Thus, the thickness of the material is not directly related to the overall wet headloss.

Wet headloss for comparison purposes may be measured in the laboratory or the field (10). It is important that the porous diffusers be allowed to soak for several hours (plastics require several months) prior to testing to assure that they are completely saturated. Since the actual headloss will be a function of the degree of water saturation in the diffusers, a slightly different curve will be obtained if the airflow is started at a low flow rate and is increased or vice versa. Standard practice is usually to purge the media at the upper flow value for a predetermined time interval (5 to 10 minutes), then record additional headloss values as the flow is decreased.

Other Characteristics

Other characteristics of porous media which may also be of importance are strength, chemical stability, heat resistance, and density. Diffusion media must be strong enough to withstand static head of water over the diffuser (when air supply is off), forces applied to media under construction, and stresses of reasonable handling and shipping. All the materials described herein are impervious to normal concentrations of chemicals used in wastewater treatment including periodic exposure to strong acid solutions used for cleaning. Temperature resistance is not a problem under normal operating conditions. Density is of concern where diffusers are to be lifted out of the tank for routine maintenance.

TYPES (SHAPES) OF FINE PORE DIFFUSERS

The nature of the majority of fine pore media (ceramic and porous plastic) is such that it can be molded into practically any shape that is desirable. There are, however, a few practical guidelines which tend to influence the design. These include minimizing vertical edges to prevent bubble coalescing, keeping the shape simple to prevent molding problems, and some restrictions of size.

Today there are four general shapes of porous bubble diffusers on the market, they include plates, tubes, domes, and discs.

Plates

The original fine pore diffuser design was a flat rectangular plate. The plates are typically 30 cm (12 in) square and 2.5 to 3.8 cm (1 to 1.5 in) thick. They are manufactured from either glass bonded silica or glass bonded aluminum oxide. The plates are installed in the tank by grouting into recesses in the floor, cementing into prefabricated holders, or clamped into metal holders. A chamber underneath the plates acts as an air plenum. The number of plates fixed over a common plenum is not standard and can vary from only a few to 500 or more. In current designs, individual control orifices are not provided on each plate.

In the early activated sludge plants (1910 to 1920s), fine pore plates were used almost exclusively as the method of air diffusion. Today, other than in some of the original plants, fine pore plates are not being installed.

Tube Diffusers

Like the plates, fine pore tubes have been used in wastewater treatment for a number of years. The early tubes were Saran wound or made from aluminum oxide and have been followed by the introduction of styrene acrylonitrile copolymer (SAN), porous plastic (PE), and, most recently, the new generation of flexible media.

All the tube diffusers on the market are of the same general shape. Typically, the media portion is 51 to 61 cm (20 to 24 in) long and has an O.D. of 6.3 to 7.6 cm (2.5 to 3.0 in). The thickness of the media is variable. Flexible membranes are very thin, commonly in the 0.5 to 1.3 mm (0.020 to 0.050 in) range. The PE media is usually supplied in a 6.3 mm (0.25 in) thickness, the SAN approximately 15.2 mm (0.6 in), and fused ceramic material in the 9.5 to 12.7 mm (0.375 to 0.5 in) range.

The holder designs for the ceramic and plastic media are very similar. However, plastics need structural reinforcing. Most consist of two end caps held together by a connecting rod through the center. For the flexible membrane diffusers, the end caps and support frame are one piece. The membrane material is installed over the support frame and clamped on both ends.

Tube diffusers are designed to operate in the 3 to 17 m^3/hr (2 to 10 scfm) range. Because of their inherent shape, it is sometimes difficult to obtain air discharge around the entire circumference of the tube. The air distribution pattern will vary with different types of diffusers. In general, the prevalence of inoperative area will be a function of the airflow rate and the headloss across the media. Because dead areas can provide sites for slime growth, it would be beneficial prior to selecting a particular design of tube, to observe its performance on a laboratory or pilot scale basis to assure that proper air distribution will be obtained at the design flow conditions.

Most tube assemblies are fitted with a control orifice inserted in the inlet nipple to aid in air distribution throughout the system. Typically the orifice is approximately 12.7 mm (0.5 in) in diameter, although different sizes can be used for various design flow rates. Also, some assemblies include check valves to prevent the backflow of liquid into the air piping.

Dome Diffusers

The fine bubble dome diffuser was developed in Europe in the 1950s and introduced in the U.S. market in the early 1970s (12). Considered as the standard in England and some parts of Europe, domes are now installed in a number of U.S. plants.

The dome diffuser is essentially a circular disc with a downward turned edge. Currently, these diffusers are 17.8 cm (7 in) in diameter and 3.8 cm (1.5 in) high. The media is approximately 15.2 mm (0.6 in) thick on the edges, and 19.0 mm (0.75 in) on the top or flat surface.
domes presently are being made only from aluminum oxide.

The dome diffuser is mounted on either a PVC or mild steel saddle type base plate. The diffuser is attached to the base plate by a bolt through the center of the dome. The bolt can be made from a number of materials including brass or stainless.

The slope of the headloss versus airflow curve for a ceramic diffuser is very flat. It has been reported that a variation from the average of ±10 in the specific permeability can result in a 200% change in airflow for the same headloss (13). To better distribute the air throughout the system, control orifices are placed in each diffuser assembly to create addition headloss and balance the airflow. The fastening bolt is hollowed out and a small hole drilled in the side or the orifice is drilled in the base of the saddle. The size of the orifice is typically 5.0 mm (0.2 in).

Dome diffusers are usually designed to operate at 1.7 m^3/hr (1.0 scfm) with a range of 0.8 to 3 m^3/hr (0.5 to 2.0 scfm). In designing a system, careful consideration should be given to the desired airflow range. Testing has shown that the oxygen transfer efficiency (OTE) is dependent on the airflow rate per diffuser increasing as the airflow rate decreases. This can lead to the temptation to design systems to operate at 0.7 to 0.8 m^3/hr (0.4 to 0.5 scfm)/diffuser. Although favorable in terms of oxygen transfer, this practice can lead to operational problems. At low airflow rates, uniform air distribution across the entire diffuser surface may be difficult to obtain. Also, at 0.8 m^3/hr (0.5 scfm), the headloss across the control orifice will be less than 25 mm (1.0 in) water. At low airflow rates, the orifice will not serve its intended purpose of balancing the air throughout the system. In any case, if either the entire area or portions of the diffuser are not discharging air, foulant development can begin which could lead to a premature fouling of the system.

The upper limit for airflow through a dome diffuser is usually considered to be 3 m^3/hr (2.0 scfm). Operation above this level is possible, but is not very economical. Increasing the flow rate results in a continued decrease in OTE and may require a larger control orifice.

Disc Diffusers

Disc diffusers are a relatively recent development. Discs are flat, or relatively so, and are differentiated from the dome diffuser in that they do not include a downward turn peripheral edge. While the dome design is relatively standard, currently available disc diffusers differ in size, shape, method of attachment, and type of material.

Disc diffusers are available in diameters which range from approximately 18 to 24 cm (7 to 9.5 in) and thicknesses from 13 to 19 mm (0.5 to 0.75 in). With the exception of two designs, all consist of two flat parallel surfaces. Although the majority of disc diffusers are made from aluminum oxide, a porous plastic (PE) disc is also available.

Like the dome diffusers, the disc is mounted on a plastic (usually PVC) saddle to type base plate. Two basic methods are used to secure the media to the holder, a center bolt or a peripheral clamping ring. The center bolt method is similar to that used with the domes. The more common method of attaching the disc to the holder is to use a screw on type retainer ring. With the threaded type collar, a number of different types may be used.

In general, the retainer ring method of attaching the diffuser to the holder has two potential advantages over a center bolt. It has been reported (15) that as the diffusers become fouled, excessive amounts of air are discharged from the edges and the area around the center bolt washer. Although not specifically documented under controlled conditions, this nonuniform airflow could reduce the OTE of the system. The retainer ring will tend to minimize these problems. A second advantage is that breakage of diffusers from over tightening the bolt or air leakage problems from stretching a nonmetallic bolt can be eliminated.

For the disc diffuser designs, there are two methods of attachment to the air piping. The first is to solvent weld the base plate to the PVC header prior to shipment to the job site. The second technique uses a mechanical method of attachment. This can be either a bayonet type holder which is forced into a saddle on the pipe or a wedge section which is placed around and clamps the holder to the pipe.

Disc diffuser assemblies also include individual control orifices in each assembly. Designs employing the bolt method of attachment usually will use a hollow bolt with a orifice drilled in its side. The other designs will either have the orifice drilled in the bottom of the diffuser holder or the base will include a threaded inlet where a small plug containing the desired orifice can be inserted. The diameter of the orifice is similar to that used with the dome diffusers.

Disc diffusers have a design flow rate of from 0.8 to 5 m^3/hr (0.5 to 3.0 scfm)/diffuser. The most economical operating range will, however, be somewhat dependent on the size. The 18 cm (7.0 in) diameter discs are usually operated in the 0.8 to 3.4 m^3/hr (0.5 to 2.0 scfm) range, similar to the dome diffusers. For the larger discs 22 to 24 cm (8.5 to 9.5 in), the typical lower limit may be 1.3 to 1.5 m^3/hr (0.75 to 0.9), up to an upper limit of 4.3 to 5.1 m^3/hr (2.5 to 3.0 scfm). Prolonged operation at flow rates less than 1.3 m^3/hr (0.75 scfm) is not desirable with a large disc because insufficient air is available to assure good distribution across the entire surface of the media. In those applications where operation above 3.4 m^3/hr (2.0 scfm) is desirable, the control orifice should be sized accordingly so that the headloss produced does not adversely affect the economics of the system.

Clean water testing has shown that the oxygen transfer efficiency is related to diffuser size (12,14). A fewer number of large diameter discs are required to achieve the same oxygen transfer results. Apparently, the additional surface area results in a lower flux rate, thus the higher transfer. There is, however, no generally accepted ratio for comparing the various size diffusers. As a range, one 23 cm (9 in) diameter disc has been found to be approximately equivalent to 1.1 to 1.4 - 18 cm (7 in) discs. The actual ratio is related to airflow rate and diffuser submergence. Since the surface areas are nearly the same, a 17.8 cm (7 in) diameter disc and dome should achieve comparable results.

AIR PIPING AND DIFFUSER LAYOUT

Fine bubble plates are normally installed in either total floor coverage or spiral roll. Total floor arrangements may include closely spaced rows (transverse or longitudinal) or incorporated in a ridge and furrow design. Total floor layouts produce higher oxygen transfer efficiencies than the more efficient mixing spiral roll.

Most tube diffusers can be attached to existing left out assemblies which make them especially suited for retrofit. They are often installed parallel along one or both long sides of the aeration basin. Newer designs, however, employ cross roll or full floor coverage patterns.

Both domes and discs are installed in a total floor coverage or grid type pattern. In some cases where mixing may control the design (near the end of long narrow tanks), the diffusers can be placed in tightly spaced rows along the side or middle of the basin to create a spiral type mixing pattern. The diffusers are usually mounted as close to the tank floor as possible, within 23 cm (9 in) of highest point being typical.

PERFORMANCE CHARACTERISTICS

In the late 1960s and early 1970s, consulting engineers began specifying clean water performance tests to be conducted by the aeration equipment suppliers as a means of verifying aerator performance. Various engineers developed their own testing criteria.

In April 1978, a "Workshop Toward An Oxygen Transfer Standard" (15) co to sponsored by the U.S. Environmental Protection Agency and the American Society of Civil Engineers was held in an effort to obtain concensus standards for the evaluation of aeration devices in both clean and process water. The outcome of the workshop was the formation of an Oxygen Transfer Standards Subcommittee under ASCE (16) and progress toward the development of process water test procedures (17).

CLEAN WATER PERFORMANCE

This section presents a distillation of clean water performance data on fine pore diffusion devices. Some but not all of the data was generated using the current ASCE recommended clean water standard (16). Thus, the oxygen transfer results presented in this section reflect the utilization of the current nonlinear least squares method of analysis as well as a prior procedure using the linear least squares log deficit analysis (16). The latter method permitted data truncation. Both methods produce comparable results under ideal testing conditions. Every effort has been made to screen the data reported herein and to omit data of questionable validity.

The results of clean water transfer tests are reported herein as Standard Oxygen Transfer Efficiency (SOTE), Standard Oxygen Transfer Rate (SOTR), or Standard Aeration Efficiency (SAE). Standard conditions are defined as clean water (alpha = 1.0, beta = 1.0), Temperature = 20°C, Atmospheric Pressure = 1.0 atmosphere and D.O. = 0.0 mg/l.

One of the critical parameters required for the calculation of oxygen transfer rates is the equilibrium D.O. saturation concentration, C^*_∞. For submerged aeration applications, C^*_∞ is significantly greater than the surface saturation value, C^*_s, tabulated in most standard tables (17). It is therefore necessary to either calculate C^*_∞ (17), or to measure it during clean water tests (16). This value is primarily dependent upon diffuser submergence, diffuser type, tank geometry, and gas flow rate. One of the more comprehensive evaluations of C^*_∞ in clean water tests was reported by Yunt et al. (18).

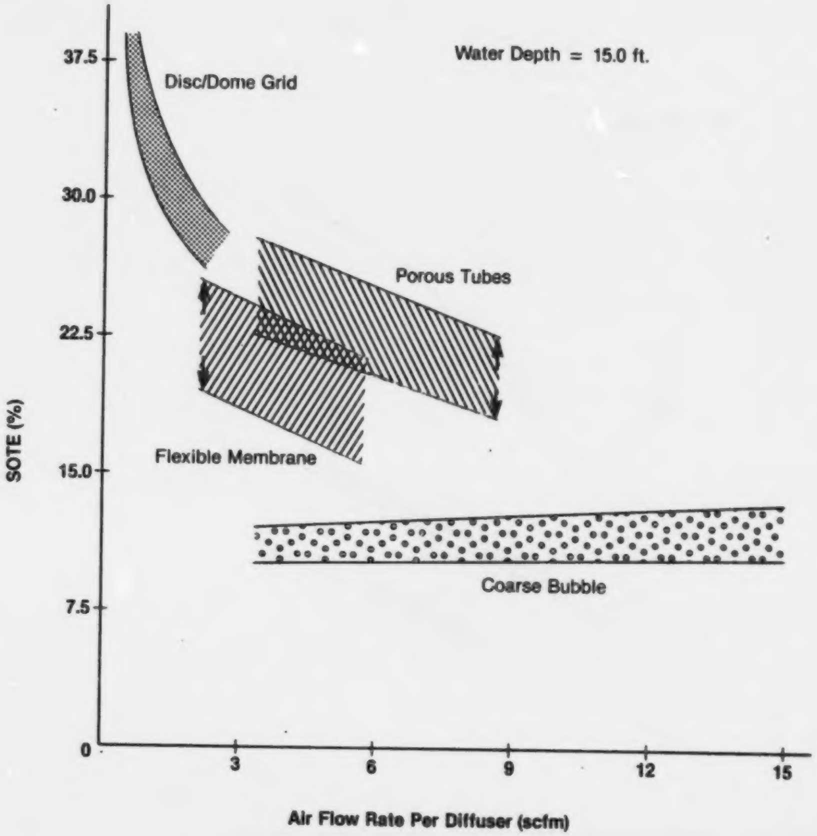
The performance of diffusers under clean water test conditions are dependent upon a number of factors in addition to those standardized in the calculation of SOTE, SOTR, or SAE. Among the most important factors are diffuser type, diffuser placement and density, gas flow rate per diffuser, and tank geometry.

Typical oxygen transfer efficiencies for fine bubble diffused air systems are presented in Table 1 and Figure 1. These data are reported for a 4.6 m (15 ft) diffuser submergence. The effect of diffuser type, placement, and airflow per diffuser are clearly delineated from this summary of eight different clean water studies. In general, it can be observed that ceramic domes and discs demonstrate slightly higher clean water transfer efficiencies than typical plastic porous media or flexible membrane tubes in a grid placement. Both tubes and discs/domes are significantly superior to all coarse bubble placements. Within a given diffuser type, spreading the diffusers more uniformly along the tank bottom area (spiral to dual or grid) tends to improve clean water performance.

TABLE 1. CLEAN WATER OXYGEN TRANSFER EFFICIENCY COMPARISON (SUBMERGENCE - 15 FT)

Diffuser Type & Placement	Airflow scfm/diffuser	SOTE - %	Reference
Ceramic Discs-Grid	0.6 to 2.9	36 to 25	14
Ceramic Domes-Grid	0.5 to 2.5	39 to 27	14,18,19,20
Plastic Porous Media Tubes			
Grid	2.4 to 4.0	32 to 28	21
Dual	3.0 to 9.7	28 to 18	14,18,22
Spiral	2.0 to 12.0	25 to 13	14,22
Flexible Membrane Tubes			
Grid	1-4	29 to 22	23
Quarter Point	2-6	24 to 19	23
Spiral	2-6	19 to 15	23
Coarse Bubbles			
Dual	3.3-9.9	13 to 12	18,24
Midwidth	4.2-45	13 to 10	18,24
Spiral	10-35	12 to 9	18,24

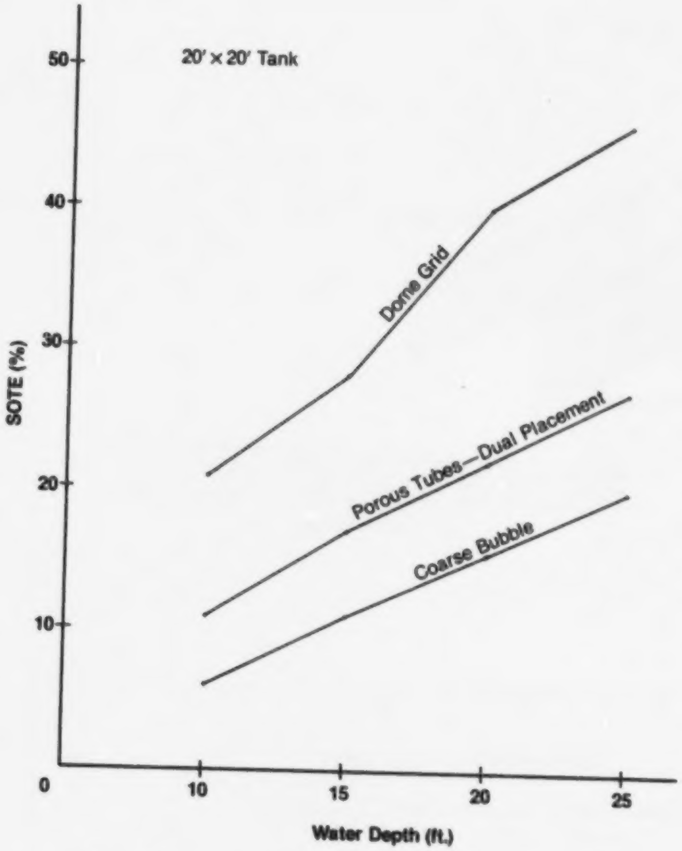
Figure 1 also demonstrates the effect of airflow rate per diffuser on oxygen transfer efficiency. Whereas domes and discs in a grid placement show a dramatic decrease in SOTE with increased airflow, plastic porous media, and flexible tubes exhibit an intermediate effect and coarse bubble patterns are relatively unaffected by gas flow rate with some indication of increasing SOTE at the higher gas flows.



Note: Higher transfer efficiency at one air flow indicates increased density or dual placement

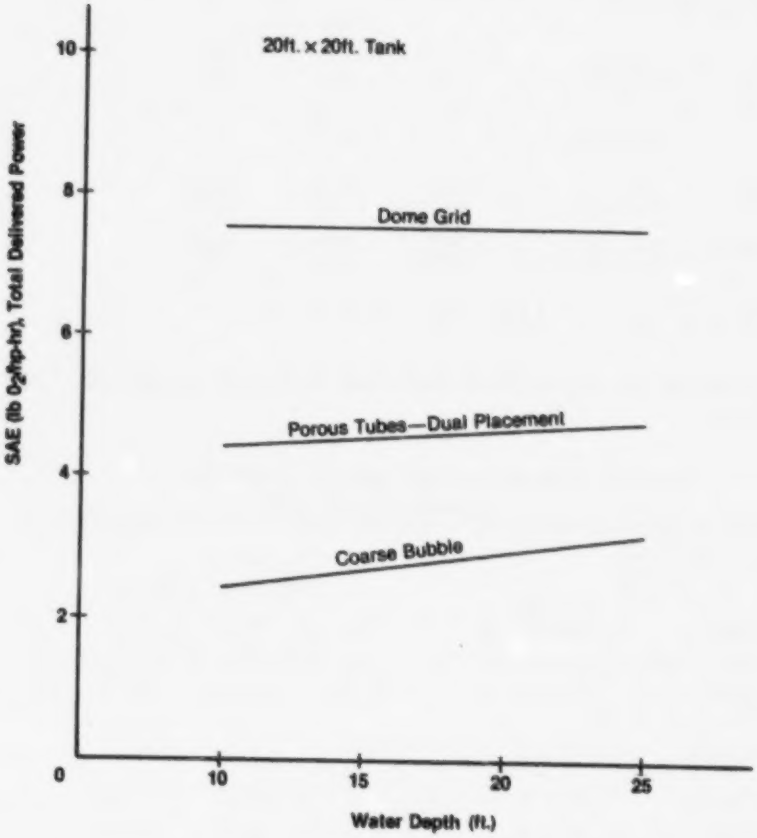
Figure 1. Oxygen transfer efficiency vs air flow rate.

The effects of diffuser submergence on aeration performance for several types of diffusers are illustrated in Figures 2 and 3. Although these data are for one specific test tank and airflow rate (18), they are generally illustrative of the effects of submergence on performance. In general, SOTE values will increase with submergence since mean oxygen partial pressures are higher (thereby resulting in a greater driving force), and there is opportunity for longer bubble residence time in the aeration tank. The SAE, however, remains relatively constant (or may decrease) for the fine bubble diffusers as depth increases since power requirements to drive the same volume of air through diffusers at the greater depths will increase. Note, however, that coarse bubble diffusers exhibit a gradually increasing SAE with submergence but never reach efficiencies achieved by the fine bubble systems.



Note: Domes at 0.5 hp/1000 cu.ft.; other systems at 1.0 hp/1000 cu.ft.

Figure 2. Oxygen transfer efficiency vs depth (4).



Note: Domes at 0.5 hp/1000 cu.ft.; other systems at 1.0 hp/1000 cu.ft.

Figure 3. Wire aeration efficiency vs depth (4).

The clean water transfer efficiencies reported in Table 2 are for polyethylene or plastic porous media tubes. The dual and spiral rolls data is from Yunt et al. (18), Huibregtse et al. (14), and Paulson (23). The grid results are from Popel (22). The tube performance is typical of fine bubble diffusers and exhibits the effect of increase diffuser density. Popel observed that increased diffuser density in grids decreases upward flow velocities and therefore increases the retention time of bubbles.

TABLE 2. CLEAN WATER OXYGEN TRANSFER EFFICIENCY - POROUS PLASTIC TUBES

Placement	Airflow scfm/diffuser	SOTE at Water Depth - %		
		10 ft	15 ft	20 ft
Grid*	2.4 to 4.0	--	32-28	--
Dual Roll	3.2 - 6.3	16-11	24-17	32-22
	9.0 - 9.7	14-10	15	26-21
Spiral Roll	2.0 - 6.7	15-12	20-15	25-22
	8.0 - 12.0	15-10	17-10	22

*7.7 ft^2/tube at 13.6 ft water depth. Tank 14.4 ft x 108.2 ft

Typical performance of a flexible membrane diffuser from Wyss (23) is presented in Table 3.

TABLE 3. CLEAN WATER OXYGEN TRANSFER EFFICIENCY - FLEXIBLE MEMBRANE TUBES

Placement	Airflow scfm/diffuser	SOTE at Water Depth - %		
		10 ft	15 ft	20 ft
Floor Cover (Grid)	1 to 4	18-14	27-21	35-29
Quarter Points	2 to 6	15-13	22-18	29-24
Center Roll	2 to 6	11-9	18-15	27-23
Spiral Roll	2 to 6	11-7	18-14	28-21

*Density 4-8 ft^2/tube

This diffuser also exhibits a decreasing transfer efficiency with increasing airflow rate. The effect of diffuser placement is also shown. The increase for quarter-point placement in a rectangular basin is greater than the mid to width placement.

The clean water oxygen transfer efficiency of disc/dome grid systems are illustrated in Table 4 and Figure 4. The results are from studies by Huibregtse et al. (14), Yunt et al. (18), Sullivan and Gilbert (20), and Paulson (21). This type of system has produced the highest transfer efficiencies reported for fine bubble devices. The density of placement is greater than the tube-grid systems and the airflow rates per diffuser are lower. Huibregtse (19) reported a slightly increased transfer efficiency with a 238 mm (9.4 in) dia disc versus a 178 mm (7 in) dia dome. Houck and lower. Huibregtse (19) reported a slightly increased transfer efficiency with a 238 mm (9.4 in) dia disc versus a 178 mm (7 in) dia dome. Houck and Boon (12) have also reported a similar relationship between dome or disc diameter and oxygen transfer per diffuser.

TABLE 4. CLEAN WATER OXYGEN TRANSFER EFFICIENCY - CERAMIC DISC/DOME GRID SYSTEMS

Diffuser Density sq. ft/diffuser	Airflow scfm/diffuser	SOTE at Water Depth - %			Ref.
		10 ft	15 ft	20 ft	
Disc-9.4"					
6.4	0.9-3.0	22-20	31	37-34	5
4.1	0.8-2.9	24-21	34-30	41-35	5
3.2	0.7-2.6	25-22	34-31	41-38	5
Dome-7"					
5.6	0.5-2.0	--	31-25	40-28	6
4.2-4.4	0.5-2.5	23-16	32-25	41-30	6,7
3.2-3.3	0.5-2.0	24-20	37-27	44-31	4,5,6
2.2-2.5	0.5-2.5	23-17	35-27	47-33	6,7
1.5	0.5-2.5	26-18	34-27	--	7

As the disc or dome density increases (decreasing area per diffuser), the transfer efficiency increases for a given airflow rate. The studies of Sullivan and Gilbert (20) show a nonlinear decrease in transfer efficiency while the results of others indicate a linear decrease in transfer efficiency with increasing airflow rate.

PROCESS WATER PERFORMANCE

In 1978, Eckenfelder opened the Workshop Toward an Oxygen Transfer Standard (15) by indicating that compared to clean water testing, "it is far more important to define aerator performance under field operating conditions." Prior to 1981, there was a general lack of consistency in the methods used to evaluate aerator performance under process conditions, and there existed a paucity of coherent data on dirty water performance. Since that time, significant data under process conditions have been developed; however, there is a continuing need to expand this data base.

Another area where coherent data are lacking in the literature pertains to measurement of alpha values for various aeration devices. A great amount of reported alpha data were obtained from bench scale units which do not properly simulate mixing and Kl_a levels, aerator type, water depth, and the geometry affects of their full-scale counterparts. As a result, much of the reported data on alpha values, particularly for diffused aeration systems is of questionable validity. Reliable full-scale test procedures for use under process conditions coupled with good clean water performance data are required to overcome these difficulties.

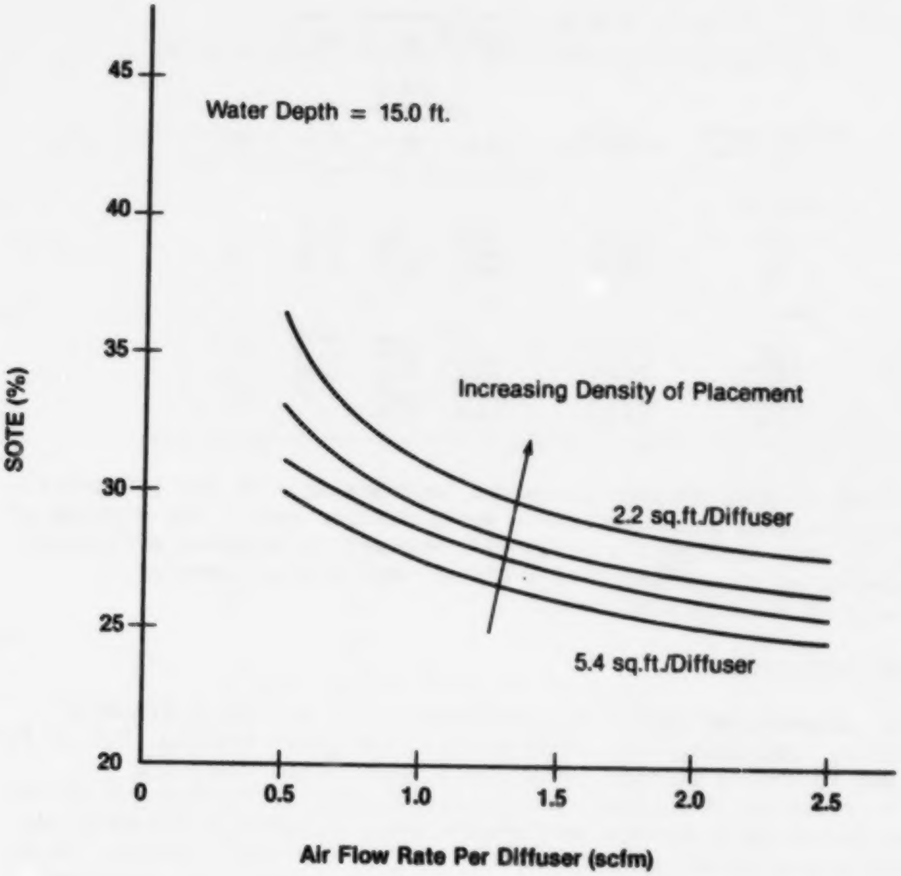


Figure 4. Effect of diffuser density on oxygen transfer.

In 1981, the ASCE Oxygen Transfer Standards Committee undertook a study to evaluate, in parallel, four principal methods for estimating oxygen transfer under process conditions at seven sites (25). These methods include the steady to state method, the nonsteady state method, an off-gas analysis procedure, and two tracer techniques. Table 5 summarizes the factors which affect the selection of the best method to use to measure process OTE values. It is the intention of this Committee to prepare a manual of methods for testing oxygen transfer devices under process conditions.

TABLE 5. SELECTED FACTORS AFFECTING OXYGEN TRANSFER FIELD TESTING FOR ESTIMATION OF OXYGEN TRANSFER EFFICIENCY (26)

FACTORS	Oxygen Transfer Tests			
	SS	NSS	OG	Inert Gas Tracers
Sensitivity To:				
Variations in -				
Influent Wastewater Flow	-	-	+	+
Oxygen Uptake Rate	-	-	+	+
Alpha	-	-	+	+
Dissolved Oxygen Concentr.	-	-	+	+
Product of (air rate x Kl_a)	-	-	+	0
Accurate Measure of -				
Oxygen Uptake Rate	-	+	+	+
Dissolved Oxygen	-	-	+	+
D.O. Saturated Value	+	+	1	+
Air Flow Rate	-	-	1	-
Other	+	+	2	3
Costs:				
Manpower	+	0	0	0
Analytical	+	+	0	-
Capital Invest.	+	+/0	0	-
Calculations:	+	0	0	0
Estd. Precision:	-	0	+	+

1 Calculate OTE directly
2 Requires accurate measurement of CO_2 in gas
3 Requires accurate estimate of ratio K_{tracer}/Kl_a - especially in diffused air systems
+ Positive response... (eg. not sensitive, less costly, more precise, easier)
0 Intermediate response
- Negative response

SS = Steady State
NSS = Nonsteady State
OG = Off-gas

The factors which affect the performance of diffused aeration systems under process conditions include wastewater characteristics, process type, flow regime, loading, geometry, equipment configuration, diffuser fouling, operational control, mechnical integrity, and preventative maintenance. In-process testing of selected diffusers under a variety of test conditions will eventually provide the data base needed by engineers to intelligently transfer standardized clean water performance data to field conditions. This data is now being collected. A partial list of that data base is presented below.

Table 6 presents process water performance data for thirteen individual evaluations of various sites employing a variety of diffused aeration devices. Each set of data represents the observed performance of a particular system over a period of several hours and is in no way suitable for the design of similar systems. The intention of this table is to give the reader a general feeling for the relative performance of the systems listed under a variety of operating conditions.

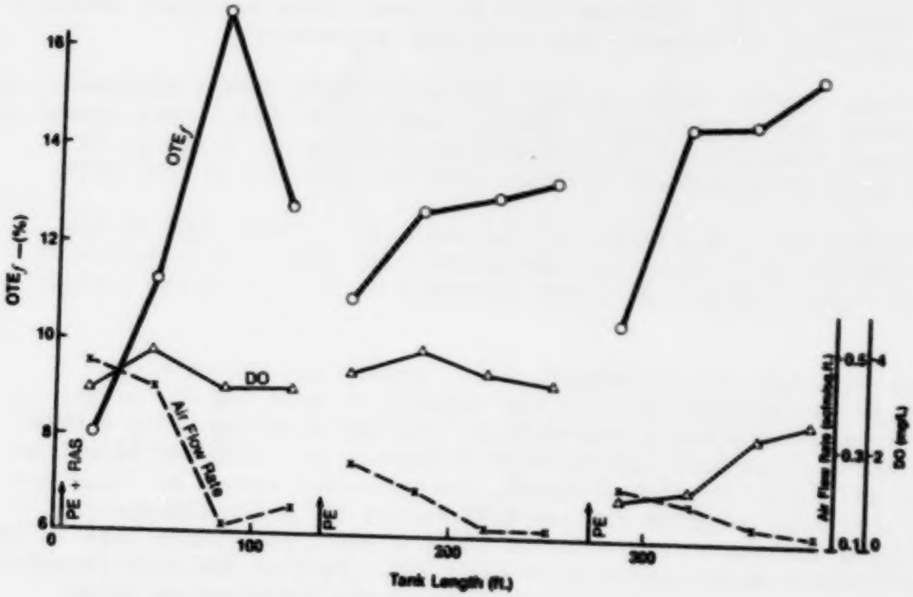
In all cases, the OTE values reported have been converted to 20°C and zero residual D.O. "Apparent" values of alpha have been estimated from clean water performance data for similar geometry, air rate per unit, and equipment placement. Since the performance of most porous diffusion devices is likely to change with time due to fouling, the term "apparent alpha" has been adopted to distinguish between the total difference in performance from clean water as compared to differences due to waste characteristics only (alpha).

The first three data sets originate from off-gas testing at Madison, Wisconsin (26,27). The ceramic grid data represents the overall performance of a three pass system.

TABLE 6. IN PROCESS OXYGEN TRANSFER DATA

Site	System	Flow Regime	In Process SOTE$_f$ (%)		Apparent Alpha		Mean Air Flux Rate (L/m^2/sec)
			Average	Range	Est. Mean	Range	
Madison, WI (26,27)	Ceramic Grid	Step Feed	17.8	12.6/26.2	0.64	.42/.98	1.42
Madison, WI (26,27)	Ceramic & SAN Tubes	Step Feed	11.0	7.5/13.4	0.62	.46/.85	2.69
Madison, WI (26,27)	Wide Band - Coarse Bubble	Step Feed	10.0	7.9/10.9	1.07	.83/1.19	0.53 2.69
Whittier-Narrows, CA (28)	Ceramic Grid	Plug Flow	11.2	9.3/15.2	0.45	.35/.60	1.07
Whittier-Narrows, CA (28)	Jet	Plug Flow	9.4	7.8/10.8	0.58	.48/.72	1.88
Brandon, WI (27)	Jet	CSTR	10.9	9.7/12.1	0.45	.40/.50	0.66
Brandon, WI (27)	Jet	CSTR	7.5	7.4/7.7	0.47	.46/.48	1.98
Orlando, FL (29)	Wide Band - Coarse Bubble	CSTR	7.6	6.8/8.4	0.75	.67/.83	4.67
Seymour, WI (29)	Ceramic Grid	Plug Flow	16.5	12.0/18.8	0.66	.49/.75	0.35
Lakewood, OH (29)	Ceramic Grid	Plug Flow	14.5	12.4/15.9	0.52	.44/.57	0.71
Lakewood, OH (29)	Ceramic Grid	Plug Flow	8.9	7.0/11.1	0.31	.26/.37	0.46
Brewery (29)	Ceramic Grid	CSTR	14.2	12.5/15.2	0.37	.32/.37	1.52
Brewery (29)	Static Aerator	CSTR	7.4	5.7/8.8	0.50	.36/.51	2.69

Figure 5 illustrates local performance in terms of OTE, air flux rate (flow per unit surface area of tank), and residual D.O. as a function of tank length. At this facility, alpha appeared to vary from about 0.4 at the inlet to near 1.0 at the discharge point. Note the reduction in apparent alpha at each point of primary effluent addition. The second data set for ceramic and SAN plastic tubes applied in a dual spiral roll configuration represents performance for the first pass of a three pass system. Passes two and three are represented by the third data set employing wide band coarse bubble diffusers also in a dual spiral roll placement. The higher relative alpha of the latter system is strongly affected by its favorable position at the rear of the process where significantly higher alphas are encountered compared to the inlet end of this facility.



Note: PE = Primary Effluent
RAS = Return Activated Sludge

Figure 5. Gas transfer analysis along tank length floor coverage, ceramic domes, tapered air (17).

The two data sets for Whittier-Narrows, California, were obtained in August 1981 after approximately nine months of operation (28). The two systems presented were part of a three system process water evaluation, conducted by Los Angeles County Sanitation District for the U.S. EPA in parallel trains. This evaluation compared the performance of a ceramic grid system to that of a jet aeration system, wherein the jets were installed on one side of the basin along the entire tank length, with the nozzles being directed across the basin, similar to a spiral roll.

It is of interest to note that the mean apparent alpha of the jet system was approximately 1.3 times that of the grid system. The terminal apparent alpha at the Whittier-Narrows facility was approximately 0.7 versus almost 1.0 at Madison. The presence of nonbiodegradable surfactants is one explanation for the low apparent alphas at the California site. This observation points out the danger of extracting data of this type for design purposes. Each treatment facility has unique characteristics, which must be considered individually.

The Brandon, Wisconsin, data depicts performance of a 9.1 m (30 ft) long by 4.6 m (15 ft) wide by 4.6 m (15 ft) deep complete mix aeration tank using jet aerators at two different airflow rates. This municipal facility treats a combination of domestic and industrial wastewater.

The Orlando system, which employs wide band coarse bubble diffusers, is included because the system treats domestic wastewater only. This system is currently being retrofitted with a ceramic grid system in an effort to improve aeration efficiency and increase aeration capacity at this site.

Data from Seymour, Wisconsin, a site analyzed by Houck (30) in his North American survey of disc and dome systems and also studied by Vik et al. (31) was lightly loaded at an SRT in excess of 25 days at the time of the tests.

The two data sets from Lakewood, Ohio, demonstrate the relative performance of two parallel basins, one recently cleaned and the other operating for approximately a year with no diffuser cleaning. The entire system was retrofitted with ceramic disc diffusers in a grid configuration during 1982 and 1983. In this instance, the uncleaned system as found was performing at a mean weighted SOTE of 8.9% versus 14.5% for the cleaned system. During the operating period of about a year, it appears that in to process performance deteriorated by roughly 40%. Part of the deterioration in performance may be due to several periods of air interruption which occurred during retrofit.

The last two data sets provide information on both a ceramic grid and a static tube system that were tested within the same complete mix basin. Of interest is the relative performance of both systems as it pertains to rela to tive gas phase efficiency and apparent alphas. The ratio of alpha of the static tube system to that of the ceramic grid system was observed to be 0.50/0.37 or 1.35. The ratio of field OTEs at zero D.O. are roughly 1.9 to 1.

Table 7 presents data from a field evaluation of selected tubular diffusers at Madison, Wisconsin, within the last pass of a three pass system employing a dual spiral roll configuration (26). Since alpha approached unity at this location, direct use of the data is not suitable for design purposes. The relative performance of new and used ceramics and the SAN plastic tubes is interesting. The used ceramic and SAN plastic tubes were in service continuously for about three years in a different tank prior to relocation for this test. It should be pointed out that analysis of multiple systems within a given tank cannot be conducted by any other technique than off-gas analysis. Known data on tube systems and membrane systems is scant.

The data described above represents a diverse cross section of in-process diffuser performance under a variety of conditions. No attempt has been made here to correlate performance to loading, process criteria, wastewater characteristics, etc. It is evident that several gaps in our current knowledge still exist for which additional indepth study is needed to address designer concerns.

TABLE 7. IN-PROCESS TUBULAR DIFFUSER COMPARISONS WITH OFF GAS PROCEDURES AT MADISON, WISCONSIN (27)

Diffuser	D.O. (mg/1)	Mean SOTE$_f$ (%)
Wideband Fixed Orifice	0.9	8.29
Flexible Membrane Tube	1.7	14.18
Used Pearlcomb Tube	1.7	11.33
Wide Band Fixed Orifice	2.0	10.28
New Ceramic Tube	1.7	15.96
Used Ceramic Tube	1.7	11.00
Wide Band Fixed Orifice	1.2	8.29

On the basis of the data presented in Table 6, it appears that the relative alpha values between ceramic grid and other more turbulent systems such as jets, static tubes, and fixed orifice systems may not be as great as previously reported in the literature (32). In addition, the overall average apparent alpha values presented in Table 6 and elsewhere (7,33,34) appear lower than many that are normally used for design purposes.

Another major factor affecting aerator performance in wastewater is system loading and effluent criteria, especially with respect to nitrifying and non-nitrifying aeration designs. A recent study at Rye Meads, U.K., (35) exemplifies the impact of process goals relative to aeration efficiency. In this study optimization of the nitrification process in conjunction with an anoxic zone, tapering the aeration system to meet oxygen demand, and the use of D.O. to control airflow to the system resulted in overall transfer of 2 kg O_2/KWh versus 1.2 kg O_2/KWh for an unmodified control basin. A third parallel train employing tapered air and D.O. control in a non-nitrifying operational mode averaged about 1.4 kg O_2/KWh during the study phase. Both the proper dispostion of ceramic diffusers and automated D.O. control were identified as essential elements of aeration efficiency optimization.

Another factor of concern was observed in a recent long-term study of ceramic grid systems (36) where it was observed that the slope of log OTE versus log applied air rate under process conditions had a significantly steeper negative slope with increasing air rate than observed under clean water conditions. Other investigators have not observed this phenomenon (35). It is evident that generalizations on this characteristic cannot be made due to the variable nature of the phenomenon under a multitude of operating conditions. It is an area worthy of additional study.

MAINTENANCE CONSIDERATIONS

One important factor that will influence the selection of a diffuser system for new or retrofit facilities deals with system maintenance requirements. A major concern voiced regarding fine pore diffused aeration is the fouling of the diffuser and the impact of fouling on performance.

DIFFUSER FOULING

Porous ceramic diffusers, introduced in the U.S. in the 1920's were the predominant air diffuser at mid-century (5,7). Types of fouling were identified by early investigators and the list expanded by recent investigations to include (10).

Air Side

1. Dust and dirt from unfiltered air
2. Oil from compressors or viscous air filters
3. Rust and scale from air pipe corrosion
4. Construction debris due to poor cleanup
5. Wastewater solids entering through diffuser or pipe leaks

Liquor Side

1. Fibrous material attached to sharp edges
2. Inorganic fines entering media at low or zero air pressure
3. Organic solids entering media at low or zero air pressure
4. Oils or greases in wastewater
5. Precipitated deposits, including iron and carbonates
6. Biological growths on diffuser media

The rate of fouling was typically gauged by the rise of back pressure in service. Since significant microbiological fouling can take place with little attendant rise of back pressure, this provided a crude and qualitative measure at best.

It was common practice at that time to operate a number of diffusers off a common plenum. This resulted in less uniformity than is obtained today under present day practice of individual flow control. The lack of uniformity probably augmented the rate of microbiological fouling.

In the sixties and early seventies, the relative cost of energy to maintenance labor was low. As a consequence, many of thoseinstallations were replaced with less efficient fixed orifice diffusers. In the middle seventies, this trend was reversed and many of those installations were replaced by porous media diffusers with individual air controls.

In the early eighties, better methods of measuring the degree of fouling and the effects of cleaning became available. These methods include dynamic wet pressure, bubble release vacuum, the ratio of one to the other, and chemical, as well as microbiological analysis. The practice of employing pilot diffusers which could be removed from the tank and individually analyzed came into use (9,10).

Concurrently, better methods were developed to measure the performance of operating systems which permitted better appraisal of the effects of fouling, facilitating better scheduling and maintenance. These methods included the use of gas tracers, off-gas analysis, dual nonsteady state with peroxide, and D.O. and respiration rate profiles (17,25,27).

It is not surprising, however, that with respect to a complex phenomenon such as fouling, much remains to be learned in spite of the work that has been done. On the other hand, a number of aspects are better understood and a number of hypotheses have been developed that better explain the observed effects than heretofore possible; a general discussion of some of these follow.

One type of fouling that has been observed leads to a significant increase in headloss across the diffuser wherein fouling rates appear to be greater in the regions of high localized flux rates (operating pores). Examples of this type of fouling are believed to be air side particulate fouling and water side precipitate fouling such as calcium carbonate and iron hydroxide. This type of fouling appears to be typically accompanied by an increase in back pressure that may be greater than the capabilities of the air supply system. This may further result in insufficient air for process requirements.

It is also of interest that with this type of a relationship between air flux rate and fouling rate, increases in back pressure can be accompanied by improvements in transfer efficiency (10). Figure 6 is an idealized plot of DWP and OTE versus time with a foulant of this type.

It appears that another type of fouling exists that can lead to significant reductions in oxygen transfer efficiency with modest, if any, increases in back pressure. There is some evidence to suggest that this type of fouling tends to take place in areas of relatively low flux rates (nonoperating pores), such as the underside of tubular diffusers and along the less pervious areas of planar diffusers. As fouling progresses, flux rates in the more pervious areas are believed capable of increasing to a level at which a number of diffuser pores function in concert. This serves to reduce the component of resistance to flow provided by surface tension, and correspondingly increases the component provided by frictional resistance to flow through the porous media. It is believed that the net change in resistance may be either positive or negative. Since the fouling occurs at a greater rate in areas of low flux rate, in time, the uniformity of air distribution is imparied as is the oxygen transfer efficiency. An example of this type of fouling is believed to be the progressive growth of microbiological slimes in areas of low flux rates (10,29). Figure 7 is an idealized plot of DWP and OTE versus time with this type of foulant.

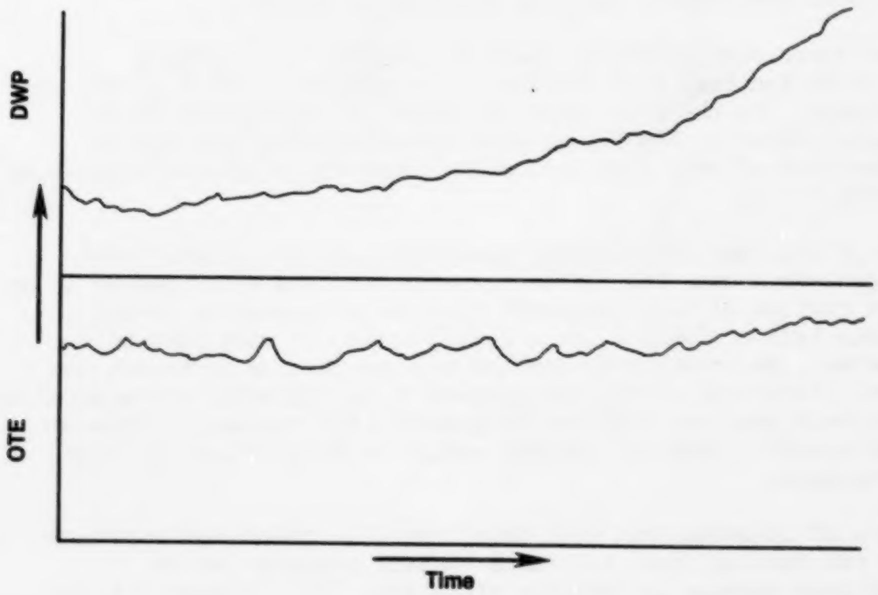


Figure 6. High local flux rate fouling.

The variables that appear to affect the rate of biofouling are not fully understood. Experience and test data (10,29) give some indication that the rate of biofouling is increased by operation at high organic loading (expressed on either a volumetric or per unit biomass basis) and/or low air rates. Some experience indicates that the rate of biofouling may be accelerated by the presence of certain types of soluble industrial wastes, particularly high strength, readily biodegradable, or nutrient deficient ones (37,38).

Other types of fouling can also be factors. Air system interruptions can allow inorganic and organic solids to enter the diffusion medium through the diffuser surface. There is no unanimity of opinions as to the consequence of this type of fouling. Fouling of the air side of diffusers

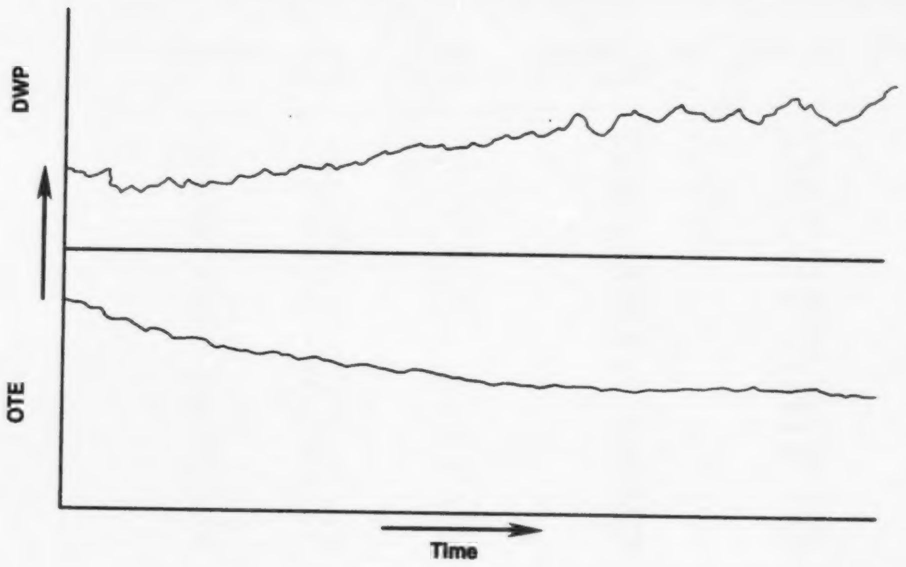


Figure 7. Low local flux rate fouling.

by the presence of mixed liquor in the air system can be serious but is considered to be preventable through design and specification.

The origin of many of the foulants which have been determined by analysis are understood. One exception is silica, which as been found in quantity at a number of sites (29,38).

It is believed that under service conditions, all the types of fouling discussed about and some others in addition can occur singly or in com to bination and with variable dominance from plant to plant and within the same plant from time to time.

A good deal of data is available and is in the process of being assembled regarding fouling and its effects. Unfortunately, consistent methods of reporting have not as yet been developed.

Table 8 is a compilation of fouling rate data from a number of ceramic diffused air municipal plants considered representative of experience today (29).

TABLE 8. REPRESENTATIVE CERAMIC FOULING DATA - MUNICIPAL PLANTS (29)

PLANT	DIFFUSER	TIME OF EXPOSURE (Days)	ΔBRV (in/yr)	ΔDWP (in/yr)	ΔDWP/ΔBRV
A	Disc	120	9.1	6.1	.67
A	Disc	120	17.3	5.8	.33
A	Disc	133	8.2	3.3	.40
A	Disc	360	7.6	3.8	.51
A	Disc	90	20.0	17.4	.87
B	Disc	365	10.5	2.2	.21
C	Disc	365	17.0	4.0	.29
D	Dome	1100	10.3	3.7	.36
E	Disc	210	20.6	8.2	.40
G	Disc	93	-	32.6	-
G	Disc	93	-	19.6	-
K	Dome	210	13.9	7.3	.53
K	Dome	210	10.8	11.1	1.01
L	Dome	360	4.8	2.3	.48
L	Dome	360	6.7	1.8	.27
M	Dome	350	6.0	2.5	.42
M	Dome	350	13.5	5.1	.38
N	Disc	900	25.5	13.0	.51
N	Disc	900	77.8	50.3	.65

Table 9 presents a similar data base from industrial origin (29). The fouling rates were calculated from the measurements made assuming a linear increase with time. Since the true rate is not usually constant, the values presented are not directly comparable, but are presented to give general orders of magnitude.

It should be noted that foulants at plants A, M, N, H1, and J1 were known to contain higher than usual fractions of inorganic constituents as well as higher ΔDWP/ΔBRV ratios.

TABLE 9. REPRESENTATIVE CERAMIC FOULING DATA - INDUSTRIAL (29)

PLANT	DIFFUSER	TIME OF EXPOSURE (Days)	ΔBRV (in/yr)	ΔDWP (in/yr)	ΔDWP/ΔBRV	INDUSTRY TYPE
AI	Dome	720	141	22.6	.16	Pulp/Paper
BI	Disc	120	59.3	18.6	.31	Pulp/Paper
CI	Disc	16	962	132	.14	Pulp/Paper
CI	Disc	92	341	56.5	.17	Pulp/Paper
DI	Disc	218	35.3	5.3	.16	Pulp/Paper
DI	Disc	110	83.4	18.3	.22	Pulp/Paper
EI	Disc	21	393	128	.33	Munic/Ind.
GI	Disc	34	280	75.2	.27	Pharm.
GI	Disc	31	73.0	24.1	.33	Pharm.
HI	Disc	420	39.1	32.0	.82	Food
HI	Disc	420	53.0	37.4	.71	Food
JI	Disc	90	219	186	.85	Brewery
JI	Disc	90	270	194	.72	Brewery
KI	Plate	30	1470	-	-	Munic/Ind.
KI	Plate	77	208	17.2	.10	Munic/Ind.
KI	Plate	58	30	-	-	Munic/Ind.
LI	Dome	110	83.4	18.3	.22	Munic/Ind.

Based on the preliminary work done to date, the following tentative observations may be proposed:

- Municipal plant fouling rates appear to be variable from plant to plant
- Fouling rates are also widely variable both spatially and temporally.
- Industrial plant fouling rates were higher and somewhat more variable than municipal plants.
- No significant or persistent differences in fouling rates were observed to be attributed to the shape or composition of the ceramic diffusers tested.
- There are possibly differences in fouling between the plastic and glass bonded ceramics since their affinity for water (and contact angle) differ as well as the relationship between bubble release vacuum and pore diameter.
- Current work tends to support earlier belief that the major factors contributing to liquid side fouling are - high organic load; low local flux rate; relatively small pore diameter.
- Airside fouling has not been found to be a significant factor in fouling in the 50 plants studied to date.
- Visual appearance of foulant has failed to consistently provide reliable basis for identifying the nature or origin of fouling.

- In plug flow systems fouling rates are usually greatest at the influent end.

- No comparative data is available on fouling rates between plug flow and completely mixed systems.

- Either inorganic or biological fouling can produce reductions of OTE by 50 percent in a matter of weeks under extreme conditions of fouling. In other situations little reduction in OTE may be noted over an extended period of time.

- Silica has frequently been found as a major constituent of diffuser foulents.

- Ferrous sulfate (and, likely other metallic salts), added to aeration tanks for process reasons may aggravate fouling.

- Most diffusers that have been tested can be restored to substantially original conditions by selected in to situ cleaning methods including hosing, steam cleaning, gas cleaning, and hose to acid cleaning.

- Little data is available yet on flexible membrane fouling.

SYSTEM MAINTENANCE

An important element in system maintenance is process monitoring. Air side and liquid side fouling of the type favored by high air flux rate cause an increase in the headloss through the diffuser at constant airflow rate, and such increases in headloss may be detected by operating conditions in the air supply system. Depending on the specific design approach, increases in the pressure in the air supply system (monitored, for example, in the blower discharge header or by increased opening of the flow control valves) indicates an increase in diffuser headloss. These factors, along with the airflow rate, should be monitored on a daily basis.

While overall system monitoring provides an indication of extreme fouling, it does not provide a very sensitive indication of increased headloss nor does it necessarily indicate significant fouling of the type inversely effected by air flux rate. For example, a 10 percent increase in system headloss (an apparent minor increase in total system pressure) may represent a significant increase in diffuser headloss. And more importantly, can indicate fouling that may have had a significant adverse effect on OTE. Moreover, fouling of only a portion of the diffusion system may lead to a significant redistribution in airflow but little increase in system pressure. Consequently, use of a more sensitive technique may be desirable or necessary. This is provided by measuring the dynamic wet pressure (DWP)(9,10).

Individual diffusers are outfitted with a series of manometers that allow measurement of the headloss across the air distribution control orifice and across the diffuser. The headloss across the orifice allows determination of the airflow through the diffuser, while headloss across the diffuser indicates the degree of fouling. By outfitting individual diffusers throughout the system, the conditions of various portions of the diffusion system can be monitored.

Since transfer efficiency can be significantly reduced by fouling without attendant significant increases in back pressure, effective monitoring will include other parameters in addition to headloss including OTE and bubble release vacuum (9). Savings in power obtainable by optimizing cleaning schedules are believed to frequently justify the modest equipment and labor required for such monitoring. A number of other candidate parameters for monitoring efficiency exists. One such parameter is the specific airflow, which is the volume of air supplied per unit of pollutant removed. Airflow is measured in standard cubic meters (standard cubic feet), while pollutant loadings are measured in terms of the BOD removed plus the ammonia transformed to nitrate-nitrogen in the plant effluent. The ratio (m^3Air/kg pollutant transformed) should be monitored, along with the aeration basin D.O.. Either an increase in the specific airflow, a decrease in D.O., or both indicate a decrease in OTE. OTE can also be measured directly using a variety of techniques previously described earlier. One of the easiest is the off-gas technique (26).

These quantitative measures of system performance should also be coupled with visual observations of the system. The surface pattern on the aeration basin should be smooth with no air "boils". These arise because of breaks in the air supply piping that allow large quantities of air to exit the system. Such leaks should be repaired as quickly as possible, both because of the decrease in OTE due to the release of coarse bubbles and because of the possibility of further damage to the diffusion system.

The uniformity of the surface pattern may indicate plugging of a portion of the diffusion system. An unusually low degree of surface turbulence in a portion of the aeration basin may indicate that the diffusers are partially fouled, thus restricting airflow to that portion of the basin. Cleaning of the diffusers in that portion of the basin may be required.

The size of the air bubbles exiting the aeration basin also provide an indication of fouling, particularly loosely adherent biomass that may cause the formation of large bubbles. Typically some degree of "coarse bubbling" is observed at the inlet end of an aeration basin, generally thought to occur because of large bubble formation as a result of high local flux rates caused by surfactants contained in the influent wastewater. These materials are quickly absorbed and/or degraded by the activated sludge, however, which restricts the size of the "coarse bubble" zone. On the other hand, if biological fouling occurs, the coarse bubble zone can expand until, in the worst cases, it covers the entire surface of the aeration basin. It is desirable that the surface of the aeration basin be inspected when initially placed in service so as to become familiar with the size and appearance of the fine and coarse bubbles exiting the inlet and outlet portions of the aeration basin. This familiarity will provide a basis for recognizing coarse bubbling, should it occur later.

A major finding of the studies of dome/disc plants in England and Holland by Houck and Boon in 1981 (12) was that the exellent O&M performance of these grid systems was due to both the knowledge and diligent care of treatment plant operators. Routine draining, tank and grid washdown, and hardware checking was standard operating procedure at all plants surveyed. Operators were also aware of symptoms of problems in the diffuser system and were quick to respond.

Preventative maintenance is necessary to keep the system in proper working order and at an optimum level of performance. Proper equipment maintenance and system operation are necessary to maintain equipment efficiency and to reduce the rate of diffuser fouling. It should also eliminate the need for emergency maintenance resulting from system failure. Certain types of fouling will occur, however, requiring periodic diffuser cleaning.

Proper maintenance of the air filtration and supply system can significantly reduce airside fouling of the diffusers. Proper operation and maintenance will generally exclude atmospheric dust sufficiently to eliminate concerns over airside plugging from this source. The guidance provided by the equipment manufacturer is generally sufficient in this area. Proper maintenance will also reduce interruptions in air supply that can lead to the entry of solids into the system, as discussed above. The deposition of solids on the liquid side of the diffuser and penetration into the upper pores is also reduced.

Proper system operation can also minimize the rate of liquid side diffuser fouling. Minimum airflow rates per diffuser must be maintained to prevent the deposition of solids that can later penetrate and plug the surface pores of the diffuser (12).

Experience indicates that these approaches will be successful in reducing the rate of liquid side diffuser fouling. However, fouling will still occur (although at a lower rate), and the diffusers must be cleaned periodically. Diffuser cleaning may be accomplished according to a regular preventative maintenance schedule that balances the cost of diffuser cleaning against the power cost savings resulting from higher system OTE (resulting in lower airflow requirements) and lower system pressure.

Figure 8 illustrates the concept of the optimum OTE. System power costs decrease with higher OTE due to lower system air requirements. On the other hand, the cleaning costs required to maintain a certain average OTE increase as the target OTE increases. This results because an increased cleaning frequency and, perhaps, a change in the cleaning method will be required to maintain a higher OTE. The optimum OTE is the one that minimizes the sum of the power cost and the cleaning costs required to maintain a higher OTE. The optimum OTE is the one that minimizes the sum of the power cost and the cleaning costs required to maintain the OTE, thus

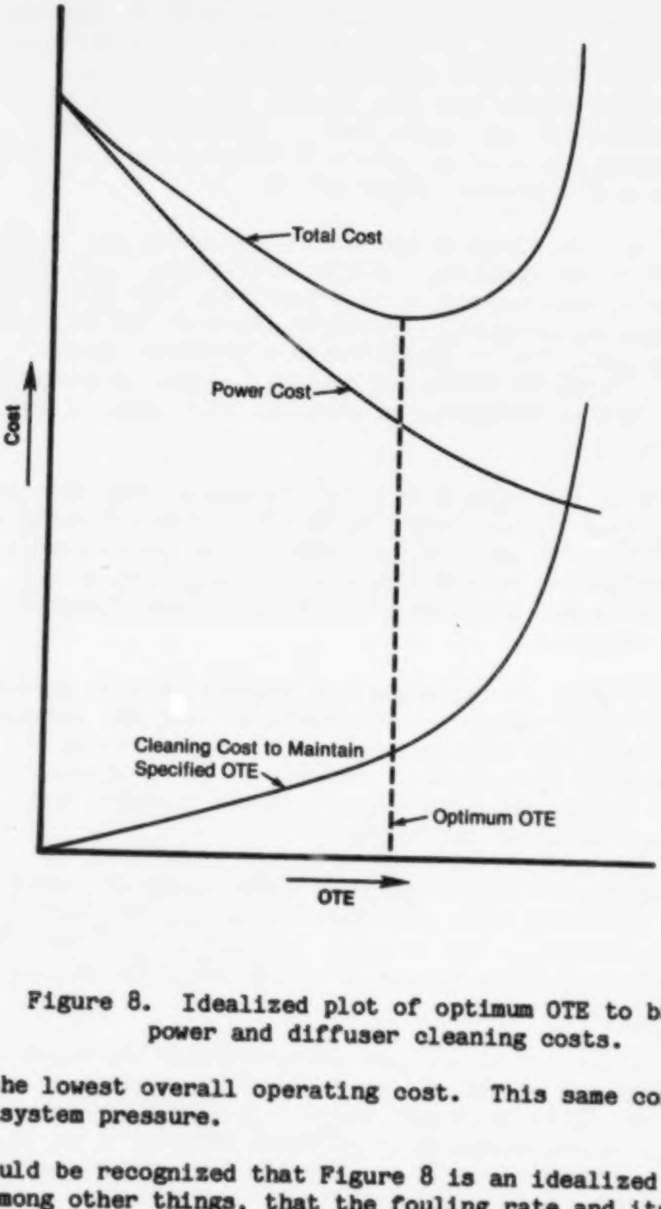


Figure 8. Idealized plot of optimum OTE to balance power and diffuser cleaning costs.

producing the lowest overall operating cost. This same concept can be applied to system pressure.

It should be recognized that Figure 8 is an idealized plot. It presumes, among other things, that the fouling rate and its effects remain constant with time and that the relationship of cleaning versus OTE does not progressively change. It would therefore be most effective to regularize monitoring programs and develop cleaning intervals around set point changes in OTE or DWP.

CLEANING TECHNIQUES

A variety of diffuser cleaning techniques are currently available, and they can be broadly classified as process interruptive and nonprocess interruptive. Process interruptive cleaning techniques require that the aeration basin be taken out of service to provide access to the diffusers, while nonprocess interruptive techniques do not require such access. A further distinction is between techniques that do not require removal of the diffusers from the basin (in-situ) and those that do require diffuser removal (ex-situ). All ex-situ techniques are process interruptive, while only some in-situ techniques are process interruptive.

Among the important in-situ cleaning methods in use today are hosing, steam cleaning, and in-situ acid cleaning. Hosing with either high pressure or low pressure sprays and steam cleaning will effectively dislodge loosely adherent liquid side biological growths. The application of 14% HCl (18 Baume' inhibited muriatic acid, 50% by volume) with a portable spray applicator to each ceramic diffuser following hosing or steam cleaning and, then, rehosing the spent acid is effective in removing both organic and inorganic foulants (29,30).

Gas cleaning consists of the injection of an aggressive gas (HCl or formic acid) into the air feed to the fouled diffusers. The oxidizing agent is transported to the diffuser by the airflow where it can oxidize most types of foulants. The exception is atmospheric dust deposited on the airside of the diffuser which has not been found to be a significant source of fouling as previously reported.

Refiring is the most expensive technique and applies only to ceramic diffuser elements. It involves removal of the diffuser from the aeration basin, placing them in a kiln, and heating them in the same fashion originally used to manufacture the element. The result is an element with most foulants removed (or incorporated into the diffuser element) and essentially restored to its original condition.

Currently, the effectiveness of the various cleaning methods being used today for the variety of different foulants encountered on different fine pore media is not well documented. Furthermore, the costs for these methods are not generally available. Current research by EPA/ASCE will develop a sound data base on cleaning technology.

Several methods are available to measure the effects of diffuser cleaning on the characteristics of the diffuser. One approach is to apply the process monitoring procedures discussed above. Thus the effects are measured as a decrease in system pressure or diffuser DWP, or by an increase in OTE or decrease in specific airflow (i.e., airflow per unit of pollutant removal). Techniques can also be applied to directly measure the characteristics of individual diffusers. These include OTE (26), chemical analysis of foulants, and measures of airflow capacity of individual diffusers. These latter techniques include specific permeability and bubble release vacuum (BRV), all of which either measure the airflow at a specified applied diffuser headloss or the applied headloss required to induce airflow through the diffuser (9,38).

As discussed above, the effectiveness and costs of the various diffuser cleaning techniques is an area of active research and the development of detailed information in this area should be forthcoming. However, there is little doubt that the incorporation of an effective cleaning schedule is a necessary component of any fine pore diffused aeration preventative maintenance program.

RETROFIT CONSIDERATIONS

DESIGN

The principal advantage of placing fine pore diffusers in municipal wastewater treatment facilities is to reduce the airflow required to provide the oxygen necessary for effective activated sludge treatment. This reduction in required airflow can result in significant energy savings in operation if proper attention is given to all the system components. Energy costs are escalating rapidly. Estimates of electrical energy cost increases of 25 to 35 percent in excess of inflation by the year 1989 have been made (39). Other reasons for fine bubble retrofit include:

- Replacement of existing equipment which has reached the end of its useful life
- Increased treatment capacity required by increased influent flow and/or organic load
- Increased level of treatment required by more stringent NPDES Permit limits

Enhanced oxygen transfer capability in itself cannot change plant treatment capacity. Aeration tank volumes and consequent hydraulic detention time must be great enough to support increases in flow and/or nitrification. If air supply capacity is a limiting factor, however, fine bubble retrofit can affect plant treatment capacity.

Wastewater characteristics will effect the design of retrofit fine pore systems. In addition to oxygen demand (carbonaceous plus nitrogeneous), some wastewater constituents may facilitate diffuser fouling. Furthermore, as described earlier, surfactants will play a dramatic role in process oxygen transfer.

The operating characteristics of fine pore diffused air systems are different than those of other oxygen transfer devices, and these differences affect process design. While diffused air systems can produce strong vertical mixing components, horizontal components will generally either be unidirectional (in cases where the diffused aeration equipment is located along only one portion of the basin as in spiral roll) or largely nonexistent (in the full floor coverage example). Consequently, the flow pattern is likely to be plug flow in character above certain length to width ratios, resulting in a gradient in process oxygen demands from the aeration basin influent to effluent.

The airflow range over which a particular fine pore diffuser can effectively operate must be identified. In general, the lower limit is set by the flux rate required to maintain uniform flow across the diffuser. Operation below this value may result in accelerated fouling. The lower gassing rate must also be high enough to provide adequate suspension of the mixed liquor. The upper limit corresponds to the airflow rate beyond which a significant decrease in the oxygen transfer efficiency is observed.

Taken together, these factors generally mean that fine pore diffused aeration systems frequently are designed with tapered aeration capabilities in tanks with high length-to-width (aspect) ratios. At a minimum, the diffuser density (i.e., effective area of per unit aeration basin floor area) should be varied, with the highest density near the tank inlet and the lowest at the tank outlet. Such variations should be designed to meet expected variations in air requirements, considering both variations in process oxygen requirements and alpha factors along the length of the aeration basin. It may also be desirable to section the diffusion system into grids, with independent air supply control to each grid. A total of three grids might typically be provided in an aeration basin with a length to to width ratio of 3 to 1 or greater.

Failure to provide proper tapering in tanks with high aspect ratio or in staged tanks can result in inadequate oxygen transfer capacity at the inlet end of the aeration basin, resulting in low dissolved oxygen con to centrations. Such conditions have been found to result in accelerated biofouling of fine pore diffusers (discussed in more detail below) and may also lead to other process and/or operational problems (12). Overdesign (in terms of the number of diffusers provided) can lead to system inefficiency if the airflow rate to meet the minimum requirement per diffuser exceeds that to meet process oxygen requirements.

Most air supply blowers in municipal treatment plants are single or multistage centrifugal types, or rotary positive displacement units. The energy savings available with fine bubble diffusers result directly from a reduction in the air required to provide the process with necessary oxygen. This reduction in airflow will result in operating fewer blowers and/or operating the same blowers at different points on their performance curves.

The efficiency of both single and multistage centrifugal blowers can vary from more than 70% to less than 40%, depending on the blower itself, and the operating combination of discharge volume and discharge pressure. Estimating input horsepower for these units should always be done using the actual certified performance curves of the blowers or estimated performance curves supplied by the blower manufacturer.

Potential power savings resulting from reduced airflows with fine pore diffusers can be completely negated by a change in blower operating efficiency as a result of reduced airflow. It is, therefore, of primary importance to accurately estimate blower horsepower under the actual conditions that will be required to operate and not by using compression formulae which require an estimate of blower efficiency to determine power for a given discharge condition.

Centrifugal blower capacity should be regulated to the extent possible by throttling on the inlet side, as significant power savings are available at any duty point. Rotary positive displacement blower capacity cannot be varied by throttling for all practical purposes. Airflow to aeration can be changed only by operating more or less units, or by "blowing off" some of the air to atmosphere. Wasting air to atmosphere may reduce the actual airflow to aeration, but it will not reduce the power consumption of the blowers because the wasted air must first be compressed to the system pressure before it is discharged. Here again, it is crucial that blower horsepower be estimated using actual blower performance curves and under the anticipated actual conditions of operation because efficiency for rotary positive blowers is by no means constant from one unit to another.

The existing system of air distribution piping can in general be reused with some reservations. Because airflow rates will be reduced as a result of enhanced oxygen transfer efficiency, the size of the existing blower discharge headers and air mains which deliver air to the tanks will normally be sufficient. Depending on the type and arrangement of fine pore equipment, the individual drop pipes into the tanks may also be large enough. The air distribution system should be checked for adequacy, however, as part of the design of the project.

Because the small air passage orifices in fine pore diffusers can be easily clogged by inlet air particulates, air filtration is an important prerequisite (5,7). Air filters can be located on the inlet to the blowers or in-line in the air distribution system. Blower inlet filters will effectively remove contaminants from the outside air but will not protect the diffusers from dirt, rust, scale, or other debris which might already be in the downstream piping. It is recommended, therefore, that careful consideration be given to the use of in-line filters. In some cases, it may even be desirable to locate filters adjacent to the air drops into the aeration system so that only clean, new, corrosion-resistant pipe need be located between the filters and the diffusers. Existing piping systems composed of galvanized steel or stainless steel pipe may present little danger of present or future rust or scale particles plugging the diffusers if blower inlet filters are selected. Painted or uncoated steel or iron pipe should be used with extreme caution unless in-line filters are installed downstream.

One drawback of in-line filters is the incremental increase in blower discharge pressure required to overcome losses in the filters. This consumes some power, but the effects can be minimized by properly sizing and maintaining the filters. A good rule of thumb to minimize air filter pressure drop is to limit face velocities to 91 to 107 m/min (300 to 350 ft/min) through the filter at peak airflow rates.

ECONOMIC ANALYSIS

The factors used in the design of a fine pore diffuser system also comprise the basis for determining its economic viability. Installation of fine pore equipment should be undertaken only if a reasonable return on investment can be foreseen. The cost effectiveness of fine pore retrofit is most appropriately based on present day flow and loading to the plant rather than anticipated future increases and should consider the total present worth of the investment as well as "simple payback."

Simple payback indicates payback in years but neglects the effects of inflation and the time value of money. A more realistic approach to economic evaluation is based on the present worth of the the cost savings of the retrofit project at some time in the future. This type of analysis accounts for the fact that energy costs and maintenance costs will increase due to inflation and that the money invested in a fine pore retrofit project would provide a return if used for other types of investments, as well.

A more sophisticated determintion which would allow energy costs to increase at a faster rate than the general inflation rate can also be performed. An estimate of the energy cost inflation rate should be made in conjunction with the local power utility as this phenomenon could vary widely. As the payback period becomes longer, these analyses become very sensitive to the assumptions made for inflation and discount rates. If the apparent payback period exceeds five or six years, the assumptions used should be reviewed. If they are found to be reasonable and prudent, the economic viability of the retrofit should be scrutinized carefully.

ONGOING STUDIES

A great deal of progress has been made in the last five years to better delineate the design, testing, maintenance, and control of oxygen transfer equipment. Yet, it is clear from the discussion in this report that there are still many gaps in our knowledge of fine pore aeration systems to their performance in process water and how to translate clean water data to field conditions; the behavior of fine pore diffusers with respect to liquid side fouling; the strategies needed to maintain and control these systems in order to secure highest possible efficiency from the device.

There are a number of research programs now under way in the U.S., Canada, and the U.K. dealing with the design, performance, operation, maintenance, installation, control, and costs of fine bubble aeration systems.

The U.S. EPA has recently funded a cooperative research agreement with ASCE and the Subcommittee on Oxygen Transfer to evaluate the existing date base on the performance of fine pore diffused aeration in clean and process water and to carry out field studies at municipal treatment facilities as required to fill these perceived gaps. Diffuser cleaning studies will also be carried out at a number of installations in order to delineate factors affecting the selection of a particular method and to establish economic bases for that selection. Two manuals will also be prepared. The first, an interim guidelines report on the state to of to art of fine pore diffusion, from which this paper is derived and the second, a comprehensive design manual as fine pore aeration to be published in 1987.

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COMPOSTING PRACTICE IN THE UNITED STATES TODAY

by

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This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

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COMPOSTING PRACTICE IN THE UNITED STATES TODAY

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ABSTRACT

Current municipal sludge composting practice in the United States is described based on investigations at selected operating facilities. An inventory of over 40 municipal sludge composting facilities is presented and a breakdown of these facilities by size, years of operation, and dewatered sludge total solids content processed is given.

Operations at three aerated static pile facilities and one conventional windrow facility are described. One aerated static pile facility treats anaerobically digested sludge at a nominal loading of approximately 45 wet metric tons (50 wet tons) per day (Mg/d), while the other two process limed and unlimed raw sludge at nominal loadings of 360 and 180 wet Mg/d (400 and 200 wet tons per day (wtpd)), respectively. The conventional windrow facility treats 450 wet Mg/d (500 wtpd) of anaerobically digested sludge. Site features, sludge characteristics and loading variations, mixing procedures, active composting requirements, and procedures for drying, curing, and screening are reviewed, and successful odor control techniques are described. Finished compost quality and distribution is discussed, and operating costs are presented.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

INTRODUCTION

Interest in composting as a means of municipal sludge treatment in the United States began in the early 1970s. At that time, the Los Angeles County Sanitation Districts initiated windrow composting of sewage sludge at the Joint Water Pollution Control Plant in Carson, California, and the U.S. Department of Agriculture developed large-scale studies of static pile composting at the Agricultural Research Station in Beltsville, Maryland. Since that time, interest and activity in municipal sludge composting in the United States has increased dramatically.

This paper presents the results of an investigation of municipal sludge composting as currently practiced in the United States. The study was initiated in the spring of 1984 and was completed in September 1985.

STUDY OBJECTIVES

Objectives of the municipal sludge composting study were as follows:

1. To investigate aerated static pile and windrow composting technologies based on operating experiences at full-scale facilities;
2. To compare and contrast features of the aerated static pile, aerated windrow, and conventional windrow processes based on this experience;
3. To evaluate performance relative to design and operation, including cost;
4. To identify key problems associated with municipal sludge composting using these technologies; and
5. To define methods which have been used or are being considered to resolve these problems.

The investigation focused on three composting processes: the aerated static pile process, including the extended static pile technique; the conventional windrow process; and the aerated windrow process. In-vessel, mechanical processes were not considered in the evaluation.

FACILITIES INVENTORY

The municipal sludge composting study was initiated with an inventory of operating facilities in the United States. A summary of facilities inventoried is presented in Table 1.

premixing using a front-end loader (20 to 25 minutes) followed by fine mixing (15 to 20 minutes) with a manure spreader or rototiller. Fine mixing at another site is performed with three passes of a mobile composter. If only front-end loaders are used for mixing, careful monitoring of the mix must be performed. Observations made during the on-site investigations revealed that for the quantities of material mixed at the Columbus facility, 40 minutes was the minimum time necessary for effective mixing with front-end loaders alone.

Various mixtures at the Hampton Roads facility were independently tested for pore space and homogeneity as part of the on-site investigations at this location. The rototilling method provided the most uniform mixture, as presented below:

Mixing method	Pore space, percent	Unmixed clumps, percent
Front-end loader only	44	5 to 20
Front-end loader/manure spreader	60	10
Front-end loader/rototiller	62	5

ACTIVE STATIC PILE COMPOSTING

Effective static pile composting requires proper pile construction techniques, sufficient aeration, and adequate process monitoring and control during the 21-day active composting period. Together with a homogeneous, sludge-wood chip mixture at a moisture content less than 60 percent, these factors combine to ensure uniform pile aeration, prevent short-circuiting, and minimize heat loss for efficient stabilization and pathogen inactivation.

Static Pile Configurations

Static pile aeration system facilities and pile construction techniques will vary depending on site-specific conditions. However, generally one of two basic schemes is employed. One configuration involves placing perforated aeration piping of an appropriate length directly on a paved compost pad, after which the piping is covered with a base material, such as wood chips, 30 to 45 centimeters (12 to 18 inches (in.)) deep. Sludge-wood chip mixture is then placed on the base material in an extended pile configuration (see Figure 3), after which insulating cover material is applied. The aeration piping is connected by a manifold to a blower which provides either positive or negative aeration. Generally, one such extended pile compartment is constructed for each daily loading of dewatered sludges.

The second basic static pile configuration is similar to the first except that perforated aeration piping is placed in belowground troughs which are formed as part of the paved compost pad. The troughs are then filled with a material such as wood chips, after which base material, sludge-wood chip mixture and cover material are placed in a manner similar to the first configuration. The aeration piping is again connected to a blower via a manifold.

STUDY METHODS

Schematics for the aerated static pile and conventional windrow composting processes are presented on Figures 1 and 2. The aerated static pile process involves mixing dewatered sludge with a bulking agent, such as wood chips, followed by active composting in specially constructed piles such as shown on Figure 3. Typically, both recycled bulking agent and new (external) bulking agent are used for mixing. Induced aeration, either positive (blowing) or negative (suction), is provided during the active composting phase. Temperature and oxygen are monitored during active composting as a means of process control. The active composting period lasts 21 days, after which alternate pathways to produce finished compost may be employed.

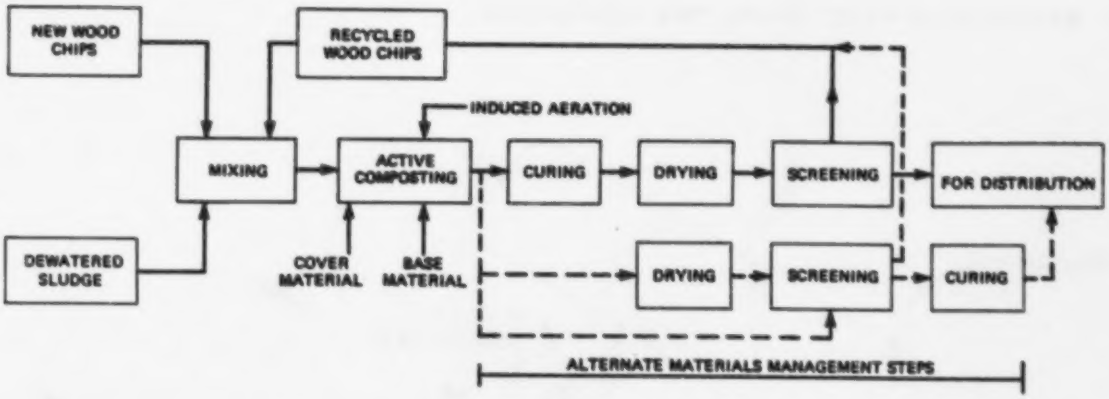
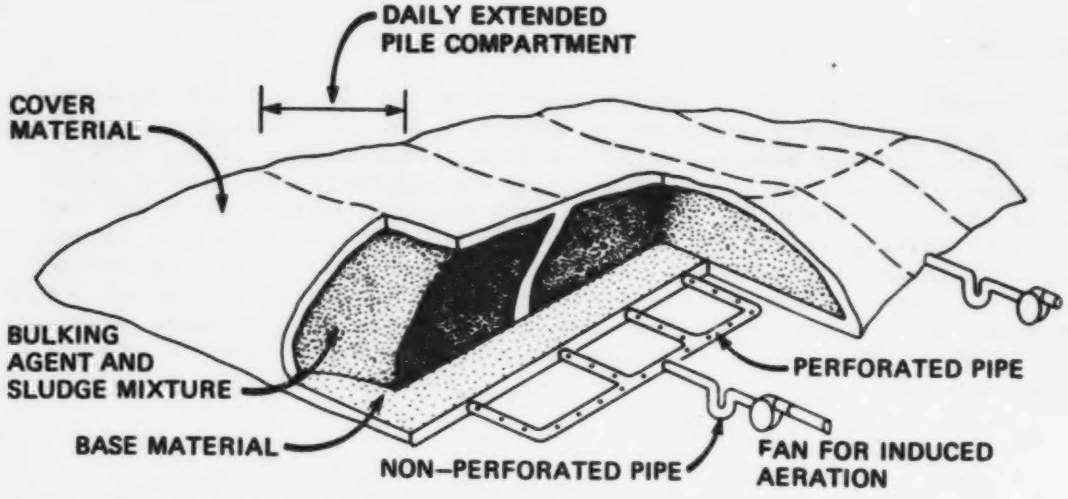

NEW WOOD CHIPS
RECYCLED WOOD CHIPS
INDUCED AERATION
MIXING
ACTIVE COMPOSTING
CURING
DRYING
SCREENING
FOR DISTRIBUTION
DEWATERED SLUDGE
COVER MATERIAL
BASE MATERIAL
DRYING
SCREENING
CURING
ALTERNATE MATERIALS MANAGEMENT STEPS

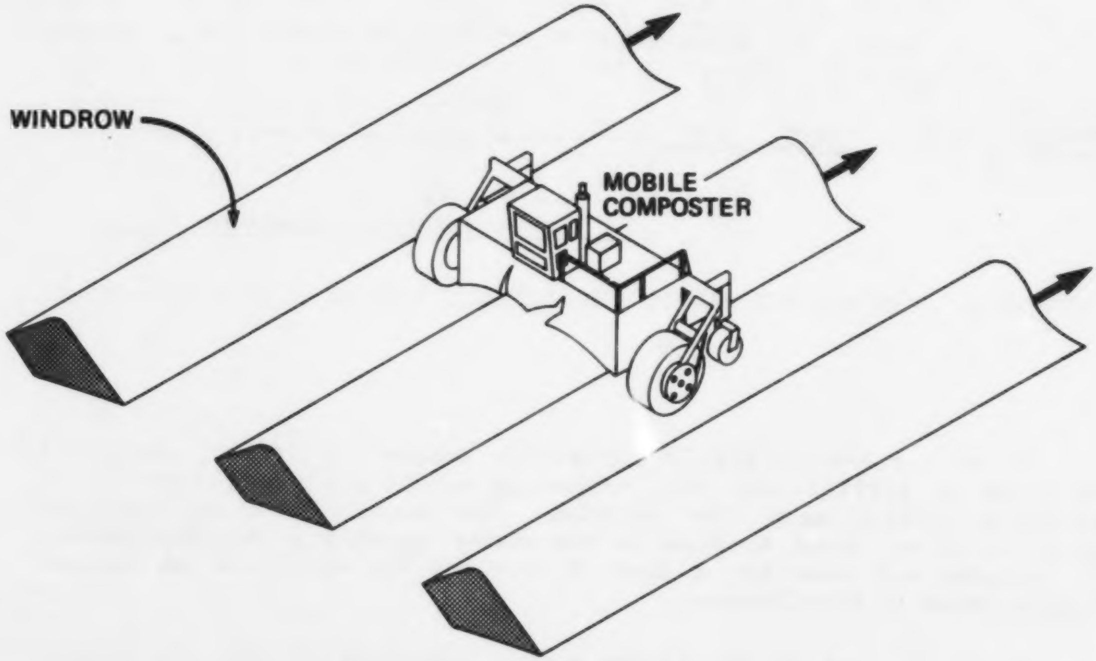
FIGURE 1. PROCESS SCHEMATIC FOR AERATIC STATIC PILE COMPOSTING

If at the end of the 21-day active composting period, composted material is sufficiently dry, screening may be performed directly to separate bulking agent for recycle. The recycled bulking agent is generally stored prior to reuse in the mixing operation. Screened compost is restacked and cured for at least 30 days and then stockpiled as finished compost prior to distribution.

If at the end of the 21-day active composting period, the compost material is not sufficiently dry for screening, a separate-stage drying step is required prior to screening, curing, and stockpiling. Alternatively, unscreened compost may be restacked for the 30-day curing period, after which separate-stage drying, screening, and stockpiling is performed.


DAILY EXTENDED
PILE COMPARTMENT
COVER MATERIAL
BULKING AGENT AND SLUDGE MIXTURE
PERFORATED PIPE
BASE MATERIAL
NON–PERFORATED PIPE
FAN FOR INDUCED AERATION

A. EXTENDED AERATED STATIC PILE COMPOSTING


WINDROW
MOBILE COMPOSTER

B. CONVENTIONAL WINDROW COMPOSTING

FIGURE 3. COMPOSTING METHODS

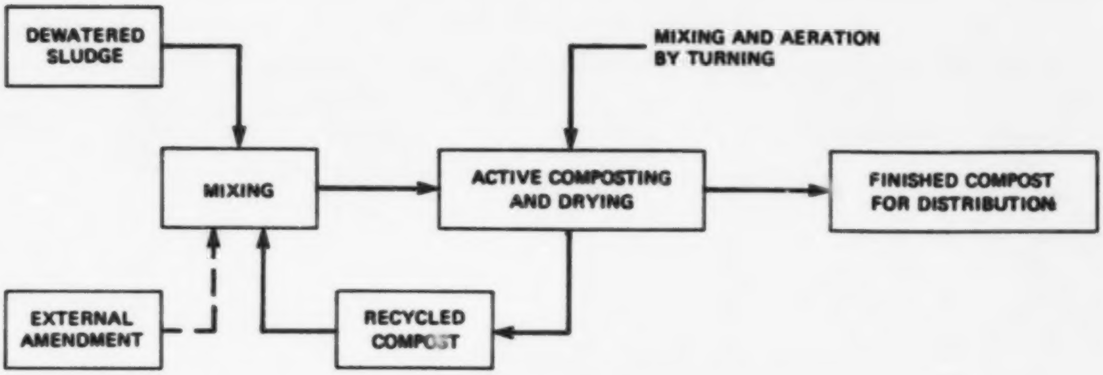

DEWATERED SLUDGE
MIXING AND AERATION BY TURNING
MIXING
ACTIVE COMPOSTING AND DRYING
FINISHED COMPOST FOR DISTRIBUTION
EXTERNAL AMENDMENT
RECYCLED COMPOST

FIGURE 2. PROCESS SCHEMATIC FOR CONVENTIONAL WINDROW COMPOSTING

The conventional windrow process (Figure 2) involves initial mixing of dewatered sludge with a bulking agent such as finished compost, often supplemented with an external amendment, followed by formation of long windrows such as shown on Figure 3. Formation of the windrows is generally performed with a specially designed, mobile composter which, in addition to giving the windrow its triangular (or sometimes trapezoidal) cross section, is capable of turning the windrow in place.

An active windrow composting period of 30 days (or more) is provided following initial mixing and formation. During this period, the windrows are periodically turned to aerate and remix the material. A turning frequency of two to three times per week is typical. Temperature is monitored for process control. Following the active windrow composting period, the composted material is allowed to cure for 30 days; then, a portion of the finished compost is recycled and a portion is stockpiled for distribution.

KEY FACILITY FEATURES

Key features of the composting facilities investigated are presented in Table 2. The Peninsula Composting Facility of the Hampton Roads Sanitation District (Hampton Roads facility), located in Newport News, Virginia, is a 45-wet-Mg/d (50-wet-ton-per-day (wtpd)) aerated static pile operation processing a mixture of anaerobically digested primary and waste activated sludge. Two facilities process raw sludge. The Washington Surburban Sanitary Commission Site II Composting Facility (Site II facility) located in Silver Spring, Maryland, and the Columbus, Ohio, Southwesterly Composting Facility (Columbus facility) in Franklin County, Ohio. The Site II facility treats limed, raw sludge at a nominal loading of 360 wet Mg/d (400 wtpd), and the Columbus facility treats unlimed, raw sludge at a nominal rate of 180 wet Mg/d (200 wtpd). The only conventional windrow facility investigated was the Joint Water Pollution Control Plant Composting Facility of the Los Angeles County Sanitation Districts (Los Angeles facility), located in Carson, California. This facility processes about 450 wet Mg/d (500 wtpd) of anaerobically digested primary and secondary sludge.

TABLE 1. INVENTORY OF OPERATING MUNICIPAL SLUDGE COMPOSTING FACILITIES, SPRING 1984

Facility charactertistics	Facility composting process		
	Aerated static pile	Conventional windrow	Aerated windrow
Total number	28	12	2
Nominal size, dry tons per day			
Less than 10	19	9	1
10 to 25	3	2	1
26 to 50	3	0	0
51 to 100	2	0	0
Greater than 100	1	1	0
Years of operation			
Less than 1	3	0	0
1 to 5	21	7	1
6 to 10	4	1	1
Greater than 10	0	4	0
Sludge total solids, percent			
Less than 15	10	5	0
15 to 20	13	5	1
Greater than 20	5	2	1

Note: Tons per day x 0.9072 = Mg/d.

A total of 42 locations composting municipal sludge were identified as of spring 1984. Twenty-eight utilize the aerated static pile process, twelve use conventional windrow techniques, and two employ aerated windrowing. Most of the facilities are under 9 dry metric tons per day (Mg/d) (10 dry tons per day (dtpd)) and have been operating about 5 years or less.

A variety of sludges are processed by the facilities, including raw, anaerobically digested, and aerobically digested sludges. Ten of the aerated static pile facilities inventoried report representative dewatered sludge total solids concentrations below 15 percent, and five of the conventional windrow systems were in this category. Thirteen static pile facilities report dewatered solids typically in the 15 to 20 percent range, although note that daily values are sometimes under 15 percent. Five of the conventional windrow and one of the aerated windrow facilities report sludge solids concentrations in this category. Eight of the municipal sludge composting facilities report dewatered sludge total solids concentrations greater than 20 percent. One of these, an aerated windrow facility, receives a heavily limed sludge having a total solids concentration of about 40 percent.

The remainder of this paper describes representative United States practice for aerated static pile and conventional windrow technologies based on investigations at three static pile facilities and one conventional windrow facility. The aerated windrow practice is not presented. Information on this technology and on other features of the municipal sludge composting investigation may be found in Reference 1.

TABLE 3. KEY SITE FEATURES

Site feature	Hampton Roads facility	Site II facility	Columbus facility
Total operational area, ac	6.2	40	37
Mixing operations			
Area, ac	0.3	1.1	0.4
Paved	Concrete	Concrete	Asphalt
Covered	Yes	Yes, enclosed two sides	Partially enclosed
Equipment	Front-end loaders with manure spreader or rototiller	Front-end loaders and mobile composter	Front-end loaders only
Bulking agent	Wood chips	Wood chips	Wood chips
Active composting			
Area, ac	1.1	2.5	3.7
Paved	Concrete	Concrete	Concrete
Covered	No	Yes	No
Aeration piping	Laid in troughs in pad	Laid on pavement	Laid on pavement
Induced aeration	Positive and negative	Negative	Positive
Drying operations			
Area, ac	1.7	0.8	-
Paved	Concrete	Concrete	-
Covered	No	Yes	-
Method	Spread and mixed on open slabs	Induced aeration	-
Screening operations			
Area, ac	0.2	1.0	0.5
Paved	Concrete	Concrete	-
Covered	Yes	Fully enclosed	No
Equipment	Mobile screens	Fixed screens	Mobile screens
Curing operations			
Area, ac	-*	3.3	4.6
Paved	No	Concrete	Concrete
Covered	No	No	No
Aerated	No	Yes	Yes
Storage operations			
Bulking agent			
Area, ac	0.4	4.5	0.9
Paved	Concrete	Asphalt	Concrete
Covered	No	No	No
Unscreened compost			
Area, ac	1.6	-	10.0
Paved	No	-	Concrete
Covered	No	-	No
Finished compost			
Area, ac	0.4	-**	0.7
Paved	Concrete	No	Asphalt
Covered	No	No	Yes, partially enclosed
Runoff collection			
Ponds	No	Yes	Yes
Area, ac	-	10.0	0.9

*Included as part of unscreened compost storage.
**Included as part of curing area.

Note: ac x 0.40469 = ha.

SLUDGE LOADINGS AND CHARACTERISTICS

Representative sludge loadings and dewatered sludge characteristics for the three aerated static pile facilities investigated are presented in Table 4. Current loadings on an operating day basis vary from 57 to 327 wet Mg/d (63 to 360 wtpd), which translate to 10 to 56 dry Mg/d (11 to 62 dtpd), based on a mean dewatered sludge total solids content, as received, of 17 percent at each plant. The number of operating days per week vary from 5 to 7. Peak-to-average day loading ratios vary from 1.4 to 1.9. Although the mean sludge total solids content at each facility is 17 percent, variations as low as 13 percent are routinely received at one and as high as 22 percent at another.

TABLE 4. SLUDGE LOADINGS AND CHARACTERISTICS

Item	Hampton Roads facility	Site II facility	Columbus facility
Type of sludge processed	Anaerobically digested primary and secondary solids	Raw, limed primary and secondary solids	Raw, unlimed primary and secondary solids
Loadings per operating day			
Average, wet tons	6.3	360	170
Average, dry tons	11	62	29
Peak-to-average ratio	1.6 to 1.8	1.4 to 1.6	1.9
Operating days per week	6	5	7
Total solids, percent			
Mean	17	17	17
Range	14 to 22	15 to 22	13 to 22
Total volatile solids, percent			
Mean	55	43	74
Range	43 to 61	34 to 51	63 to 79
Density, lb/cu yd	1,600	-	1,900
pH	8.2 to 8.3	12.0 to 12.5	-

Note: tons x 0.9072 = Mg
lb/cu yd x 0.59325 = kg/m^3

Volatile content of the anaerobically digested sludge processed at the Hampton Roads facility is typically 55 percent, with a range of 43 to 61 percent. Raw, unlimed sludge received at the Columbus facility is typically 74 percent volatile, with a range of 63 to 79 percent. Liming of raw sludge to a pH of 12.0 to 12.5 prior to composting results in volatile solids contents typically in the range of 34 to 51 percent (Site II facility).

MIXING OPERATIONS

Wood chips are used as a bulking agent at all of the aerated static pile facilities investigated. Both hardwood and softwood chips are employed, though personnel at one facility prefer hardwood chips because they do not decompose as rapidly as softwood chips and are cheaper to purchase. Chips are generally stored in piles on paved surfaces without any cover. Paved surfaces prevent wood chip losses, minimize moisture control problems during inclement weather, and keep chips clean.

Dewatered sludge is mixed with recycled and new wood chips at ratios of 3.5 to 4.5 (volume/volume) at the total solids concentrations typically received at the composting facilities studied. The mix ratio typically varies with chip quality, moisture content of sludge and chips, season, and the proportion of fresh and recycled chips used. All facilities employ mobile equipment for the mixing operation. A minimum initial total solids concentration of 40 percent in the wood chip-sludge mixture is considered essential for effective static pile composting.

For the quantities of material handled at the facilities studied, about 40 to 45 minutes is required to achieve a well-mixed material for active static pile composting. All facilities employ mobile equipment for mixing as noted in Table 3. At one facility, mixing is accomplished by

TABLE 2. KEY FACILITY FEATURES

Facility name and location	Composting process	Nominal size, wet tons per day	Sludge characteristics
Hampton Roads Sanitation District Peninsula Composting Facility, Newport News, Virginia	Static pile	50	Anaerobically digested primary and secondary
Washington Suburban Sanitary Commission Site II Composting Facility, Silver Spring, Maryland	Static pile	400	Limed, raw primary and secondary
City of Columbus, Ohio Southwesterly Composting Facility, Franklin County, Ohio	Static pile	200	Unlimed, raw primary and secondary
Los Angeles County Sanitation Districts Joint Water Pollution Control Plant Composting Facility, Carson, California	Conventional windrow	500	Anaerobically digested primary and secondary

Note: Tons per day x 0.9072 = Mg/d.

AERATED STATIC PILE OPERATIONS

SITE FEATURES

Key site features for each of the aerated static pile facilities investigated are summarized in Table 3. Total operational areas for the Hampton Roads, Site II, and Columbus facilities are 2.5, 16, and 15 hectares (ha) (6.2, 40, and 37 acres (ac)), respectively. Operational area is defined as site area actually required for composting operations, including access roads, buffer zones, and support facilities such as administrative buildings, maintenance structures, and truck scales. Storage ponds for runoff control, if required, are also included as part of the operational area. Land which is available on the site but is not a part of active operations, e.g., land available for expansion, is not included as part of the operational area. Total operational areas employed at the Hampton Roads, Site II and Columbus facilities are equivalent to 0.06, 0.04, and 0.08 ha per wet Mg/d (0.12, 0.10, and 0.18 ac per wtpd), respectively.

Areas employed for various operations vary depending primarily on facility size, materials management procedures, and process requirements. For example at the Columbus facility, a 180-wet Mg/d (200-wtpd) plant, 4 ha (10 ac) are provided for unscreened compost storage because screening operations are affected by weather, and the market for distribution of finished compost is just being developed.

Key operations such as mixing are often covered to minimize moisture control problems, and at two of the three facilities the screening operation is also protected for this purpose. Most operating areas are paved either with concrete or asphalt, in part for runoff collection and in part for moisture control and equipment access during the composting process. Equipment and/or operating methods employed at various steps vary, as noted in Table 3.

Although many facilities in the United States have been constructed with a single blower to service more than one extended pile compartment, the trend is now toward providing one blower of sufficient capacity for each daily extended pile compartment constructed. One blower may be provided to service the entire length of a pile compartment, or one may be provided at each end to service one-half of each pile compartment.

Extensive testing by plant personnel was required at two of the facilities investigated during this study to arrive at reliable pile construction and aeration system techniques for routine operation. The Site II facility constructs daily extended pile compartments which are about 34 meters (m) (110 feet (ft)) long. Each pile is serviced by one 11-kilowatt (kw) (15-horsepower (hp)) blower located at one end of the compartment which is operated in the suction aeration mode. An aeration manifold consisting of four, 15-cm (6-in.)-diameter, flexible plastic hose laterals placed at 1.5-m (5-ft) intervals on the paved compost pad extends the length of each compartment.

Each aeration lateral consists of 4.6 m (15 ft) of solid pipe at the blower end and about 26 m (86 ft) of perforated pipe. No piping is provided the last 2.7 m (9 ft) under the toe farthest from the blowers. Perforations are tapered as presented below:

Distance from blower, m (ft)	Perforations, sq cm/linear m (sq in./linear ft)
0-4.6 (0-15)	-0-
4.6-9.8 (15-32)	8.87 (0.415)
9.8-15.8 (32-52)	17.57 (0.830)
15.8-21.9 (52-72)	56.30 (2.660)
21.9-30.8 (72-101)	90.59 (4.280)
30.8-34.0 (101-110)	No pipe

This perforation arrangement has been found to produce uniform aeration during routine operations.

Base material at the Site II facility is new wood chips placed to a depth of 30 cm (12 in.), except under the toe nearest the blower. Unscreened compost base is used for a distance of 3.6 m (12 ft) at this location to reduce short-circuiting. Each 34-m (110-ft) long by 6-m (20-ft) wide by 3.6-m (12-ft) high pile compartment includes a daily 45-cm (18-in.) blanket of screened or finished compost as an insulating cover.

At the Hampton Roads facility, a 73-m by 62-m (240-ft by 204-ft) concrete composting pad is sectioned into quadrants. Each quadrent contains 12 troughs serviced by two 2.2-kw (3-hp), variable-speed blowers at each end. Each trough contains 30 m (100 ft) of 15-cm (6-in.), Schedule 40, polyvinyl chloride (PVC) aeration pipe connected to a manifold; one blower services six troughs. The pipe is perforated, with 10-millimeter (mm) (3/8-in.) holes spaced 3.8 cm (1 1/2 in.) apart, beginning 6.7 m (22 ft) from the end of the compost pile. The end of the pipe is capped.

The perforation configuration was determined from extensive testing during normal operation and provides the most uniform oxygen levels and temperatures throughout each pile. Airflow is controlled by a damper located at each trough.

Pile construction at this location includes a wood chip base, 30 to 45 cm (12 to 18 in.) deep, except at the toes, and cover material of dry, recycled (cured, screened) compost. Cover material depth is 45 cm (18 in.) in the summer and 60 cm (24 in.) in the winter. Base material at the toes is dry, recycled compost to prevent short-circuiting, and 10 to 15 cm (4 to 6 in.) of screened compost is placed on the face of the pile for added insulation and to prevent short-circuiting. Each pile is constructed to an initial depth of about 4 m (13 ft) and widths of 2.5 to 3.0 m (8 to 10 ft).

Aeration and Process Control

The Site II facility which processes raw, limed sludge applies the following typical aeration process control parameters for a 21-day active composting period:

1. First week--negative aeration at 110 to 125 m^3/h per dry metric ton (3,500 to 4,000 cubic feet per hour per dry ton of sludge (cfh/dt)) to promote material decomposition and for odor control.
2. Second and third weeks--aeration is controlled to maintain temperatures between 50 and 60 degrees C, including a minimum of 3 days above 55 degrees C to meet pathogen inactivation requirements; then, aeration is increased to 187 m^3/h per dry metric ton (6,000 cfh/dt) for drying and odor control at teardown.

Each blower used for aeration turns off for a 5-minute period every so often to enable the aeration pipes to drain. All leachate and condensate flows to drains leading directly to a sewer.

Temperature controllers are installed on each blower. The controllers are set for the desired pile temperature, and the blowers automatically turn on and run until the desired pile temperature has been reached. If the pile temperature is below its minimum temperature set point, the blower will automatically turn on in response to a timer control and aerate at a rate of at least 31 m^3/h per dry metric ton (1,000 cfh/dt) so that the pile will not go into an anaerobic state. If the pile temperature exceeds the maximum temperature set point, the controller overrides the timer and turns on the blower until the pile temperature is reduced to the set point. The timers and temperature controllers enable the site operators to maintain high oxygen levels in the piles while still maintaining desired temperatures.

Each pile is monitored at five locations for temperature and three locations for oxygen. Temperature probes are left in place during the 21 days of active composting and temperatures are recorded daily. Oxygen readings are taken daily from oxygen-monitoring tubes. A temperature and oxygen monitoring report is maintained for each pile denoting time of measurement, location, and blower mode.

During the early stages of active composting of anaerobically digested sludge at the Hampton Roads facility, airflow rate is set to maintain an oxygen content of less than 5 percent and temperatures greater than 55 degrees C within the piles. Blowers rated to 34 m^3/h (1,200 cubic feet per minute (cfm)) operate on timers. Typical seasonal operating modes are presented below:

1. Winter operation--negative aeration, cycled 10 minutes on and 20 minutes off at 31 to 34 m^3/h per dry metric ton (1,000 to 1,100 cfh/dt).

2. Summer operation--positive aeration, cycled 10 minutes on and 20 minutes off at 34 to 38 m^3/h per dry metric ton (1,100 to 1,200 cfh/dt).

For both operating modes, once 55 degree C temperatures are met for 3 consecutive days, the blowers are put on positive aeration continuously at 78 to 87 m^3/h per day metric ton (2,500 to 2,800 cfh/dt).

In the negative aeration mode, exhausted air must be treated for odors by filtering through a finished compost pile. The positive aeration mode requires no odor control; additionally, in this mode, drying of compost to 55 percent total solids is sometimes achieved during dry weather, enabling direct screening of the compost after teardown.

With the positive aeration mode, leachate does not form as it does with negative aeration. Because of this, material which collects in the aeration troughs does not get wet under positive aeration, and odor is not generated. Periodic trough cleaning is still required with either aeration mode, however.

Each daily pile at the Hampton Roads facility is monitored at six locations for temperature and at three locations for oxygen. Temperature probes are left in place during the 21 days of composting. Temperatures are recorded daily until 55 degrees C are obtained for 3 consecutive days, and then once per week. Oxygen readings are taken daily using a portable oxygen meter.

The Columbus facility uses the following process control procedure: the 0.75-kw (1-hp) aeration blowers are designed for reversible operation to blow air into or exhaust air from the compost pile. Each blower is equipped with a system to control the operation either by a timer, temperature probe in the compost pile, or manually. A timer and manual control of the blowers is utilized as required by the stage of the composting process, which changes according to the season. In winter, aeration is not started until the pile reaches 40 degrees C; then it is on for 20 minutes and off for 10. In summer, aeration starts as the pile is constructed. Positive aeration is now used. The process is monitored by the use of temperature probes that are inserted into the pile as the pile is set up. A reading is taken and recorded and this monitoring is continued until three daily readings of 55 degrees C or greater are recorded. At such time, blower rate is increased to 100 percent to remove moisture from the material. Oxygen content is not routinely measured for process control at the Columbus facility.

DRYING AND CURING

Material composted by the aerated static pile method often is not dry enough to efficiently screen directly (total solids greater than or equal to 50 to 55 percent is required). In these cases, a separate drying step is employed. As previously noted, personnel at the Hampton Roads facility found that during the summertime, when positive aeration occurs for the entire 21-day period, the compost may be dry enough for screening, and the drying step is bypassed. Screened compost is then cured to provide additional pathogen inactivation and odor removal prior to storage for distribution. Benefits of this mode of operation are immediate recycling of wood chips and reduction in the area required for curing. At other times, however separate drying is performed. Normally, unscreened compost is stored in the curing area until weather permits drying. Drying operations entail transfer of the cured compost by front-end loader to a drying slab where the material is spread out to a depth of 38 to 45 cm (15 to 18 in.). Periodically, the compost is rototilled until a minimum of 50 percent total solids is achieved. Typically, the drying time is less than 3 days in the summertime when temperatures are high. After drying, material is piled under a covered shed until screened.

Overall curing time at the Hampton Roads facility is a minimum of 30 days, as required by state regulations. Additional storage time is used, however, in both the curing area and in the final product storage area, depending on materials handling considerations.

At the Site II facility, each pile is torn down by a front-end loader after the active compost period, and composted material is transported to a drying area where it is restacked for an aerated drying step prior to screening (prescreen drying). For 24 to 48 hours prior to teardown, the piles are aerated full time, reducing temperatures to remove most of the heat. Therefore, steam or odor release is minimal. Compost is then restacked over 10-cm (4-in.) perforated pipe and connected to a 0.75-kw (1-hp) blower. The same pile dimensions as those for active composting are used for prescreen drying operations.

Constant aeration is applied and a goal of 52 percent total solids after prescreen drying has been set for process control. This can generally be met within 4 to 5 days, even under wet-weather conditions. Temperature and oxygen are monitored during prescreen drying. Generally, pile temperature increases rapidly to 55 degrees C (within 1 day) after restacking, then drops to ambient. Oxygen content is maintained at >15 percent during the prescreen drying step.

After screening, front-end loaders transport screened compost to storage for a minimum 30-day aerated curing period. Maximum storage capacity of this area is limited to a 6-month production of screened compost. Three-quarter-kilowatt (1-hp) blowers are used for curing pile aeration and aeration pipes are 10-cm (4-in.) laterals, three per blower, located on 2.5-m (8-ft) centers. Aeration maintains high oxygen levels in the curing piles and prevents release of odors. Aeration continues until the compost is distributed. Temperature and oxygen are monitored during the aerated curing period, with a similar process response as that described for prescreen drying.

SCREENING OPERATIONS

Screening is an important step in the static pile process as this operation is necessary for effective recovery and recycling of bulking agent (wood chips). Finished compost uses, common in the United States, typically require screens which produce finished compost grades in the 6- to 10-mm (1/4- to 3/8-in.) size range.

Under optimum conditions, wood chip recoveries of 80 to 90 percent can be obtained in these size fractions using the types of screening equipment employed at the three aerated static pile facilities investigated. To do so requires that the total solids content of unscreened compost be between 50 and 65 percent, depending in part on screen size and design. Exceeding the upper limit can result in losses from excessive dust generation, while not meeting the lower limit inhibits the separation of fines from the wood chips. The latter situation leads to wood chip contamination, reduced screening rates, and often mechanical problems with the screening equipment.

Under sustained routine operation, wood chip recoveries of 70 to 85 percent are typical given the heterogeneity of compost material, operational variability, and other factors. At one facility investigated, three replicate observations using a 6-mm (1/4-in.) screen at screening rates of 75 to 82 m^3/h (99 to 107 cubic yards per hour (cu yd/hr)) and an unscreened compost total solids of 56 percent, bulking agent recoveries of 64, 76, and 86 percent were obtained. By comparison, review of facility records for 3 months of routine operation showed recoveries of 42 to 93 percent, with an average of 64 percent. Unscreened compost total solids content was between 46 and 79 percent.

At the Site II facility, which employs fully enclosed screening equipment, wood chip recoveries of 85 to 87 percent can be consistently achieved during dry weather. However, under wet, cold weather conditions, screening problems occur and recoveries are less even with effective moisture control in the incoming unscreened compost.

MATERIALS FLOW CONSIDERATIONS

Representative bulk densities, total solids, and total volatile solids of materials at various steps during static pile composting are presented in Table 5. Data for facilities processing anaerobically digested sludge (Hampton Roads) and raw sludge (Columbus) are shown.

FINISHED COMPOST QUALITY AND DISPOSITION

Table 6 presents a comparison of representative dewatered sludge and finished compost characteristics at the three aerated static pile facilities investigated in this study. Trace metal concentrations in the dewatered sludge varied, and in general, this variation is reflected in the finished compost. This is also true for TKN and total phosphorus. Total volatile solids of finished compost produced from raw sludge (without lime) is slightly lower than that produced from digested sludge; finished compost bulk densities are between 535 and 900 kilogram per cubic meter (kg/m^3) 900 and 1,500 lb/cu yd).

TABLE 5. REPRESENTATIVE PHYSICAL CHARACTERISTICS OF MATERIALS DURING STATIC PILE COMPOSTING

Process conditions	Component	Bulk density, lb/cu yd	Total solids, percent	Total volatile solids, percent
Anaerobically digested sludge, summer operation	Dewatered sludge	1,544	16	54
	Wood chips			
	Recycled	769	56	71
	New	456	55	82
	Initial mix	830	41	69
	Unscreened compost	933	48	68
	Dried cured compost	875	56	59
	Screened compost	866	52	49
Raw sludge, winter operation	Dewatered sludge	1,943	16	71
	Wood chips			
	Recycled*	1,087	44	66
	New	671	58	98
	Initial mix	1,230	39	73
	Unscreened compost	1,123	40	63
	Dried/cured compost**	1,120	41	71
	Screened compost***	1,164	52	64

*Recycled unscreened compost.
**After 31 days of curing.
***Data taken from previously screened material.

Note: lb/cu yd x 0.59325 = kg/m^3

Finished compost is sold to local users at all of the facilities investigated. Demand varies with season and thus materials management procedures for low-use periods are employed. The price of finished compost varies from about \$4/m^3 to \$12/m^3 (\$3/cu yd to \$9/cu yd), depending on facility location and quantity purchased. Typical finished compost production rates are about 0.4 to 0.8 m^3 per wet metric ton (0.5 to 1.0 cu yd per wet ton) of sludge processed.

ODOR CONTROL

The "earthy" smell of stabilized compost is often detectable in the immediate vicinity of municipal sludge composting operations. Key design and operational procedures for aerated static pile facilities to minimize more objectionable odors from being generated and/or transported off site include the following:

1. Trucks used to haul dewatered sludge should be covered and cleaned frequently. This is particularly important with raw sludge.
2. Deliveries of dewatered sludge to the composting site should be managed such that mixing and other operations can be performed without sludge accumulating for long periods of time. This is particularly important with raw sludge and in hot weather.

3. The importance of moisture control during static pile composting cannot be overstressed. Whether moisture is present in incoming dewatered sludge, or the bulking agent or amendment; whether it is added during processing from inclement weather; or whether it is generated as a by-product of the composting process itself, moisture must be controlled for effective odor control as well as for stabilization, pathogen inactivation, and finished compost quality control. An initial mix moisture content of 60 percent or less (total solids $\geq$40 percent) is a key process design criterion for effective performance. Enclosing the mixing operation, or other operations, and scrubbing the exhaust gas from the aerated pile can be an effective step for odor control in some situations.

4. Initial mix uniformity and porosity are also important for odor control during the active composting period. The presence of clumps of unmixed sludge can lead to anaerobic, and thus odorous, conditions during the active composting period, as well as incomplete stabilization and pathogen inactivation.

5. Proper pile construction techniques are important to ensure porosity, aerobic conditions, minimize heat loss, and prevent short-circuiting. Construction of daily pile compartments, with cover material, is also important.

6. Positive aeration during active composting can minimize odor generation potential since the pile cover material acts as an odor scrubber. Negative aeration can require the use of a separate exhaust scrubber system such as a finished compost filter pile. Regardless of the aeration mode employed, control of aeration rate, oxygen content, and temperature is critical for effective odor control (and proper composting).

7. Pile teardown can be managed to minimize release of odors. At one facility studied, piles are not torn down during wet weather or in the early morning hours when air inversions may result in odor generation. At another, high-rate aeration for 24 to 48 hours prior to teardown has been effective in odor control.

8. An effective leachate, condensate, and runoff collection and disposal system will minimize odor generation potential. Proper drainage of aeration piping and troughs, and means of collecting and transporting leachate and/or condensate to points of disposal such that liquid does not accumulate and stagnate, are important odor control features. Proper site drainage is required to prevent ponding which can generate odor.

9. Effective housekeeping procedures such as washing equipment and flushing or sweeping working areas such as mixing pads will also reduce odor generation potential.

TABLE 6. COMPARISON OF DEWATERED SLUDGE AND FINISHED COMPOST CHARACTERISTICS

Characteristic*	Hampton Roads facility		Site II facility		Columbus facility	
	Sludge	Compost	Sludge	Compost	Sludge	Compost
Solids, percent						
Total	17	52	17	51	17	52
Total volatile	55	49	43	-	74	64
Density, lb/cu yd	1,600	900	-	1,100 to 1,500	1,900	1,100 to 1,400
Trace metals, mg/kg						
Cd	10	6.5	3.0	2.0	38	14
Cu	714	430	186	112	272	216
Cr	102	-	-	-	279	67
Pb	197	140	196	40	287	178
Ni	57	30	24	46	-	-
Hg	2.21	-	1.8	0.4	-	-
Zn	1,651	900	487	176	2,583	965
TKN, as N, percent	5.4	2.6	2.5	1.0**	-	1.3**
Total phosphorus, as P, percent	t2.8	2.1	1.2	0.6	-	3.3
Fecal coliform per 100 gm	-	<2 to 60	-	-	-	>110
Salmonella	-	Negative	-	Negative	-	Negative
pH	8.2 to 8.3	5.9 to 6.8	12.0 to 12.5	-	-	4.6 to 9.0

*Units are based on dry weight.
**Total nitrogen.

Note: lb/cu yd x 0.59325 = kg/m^3

STATIC PILE OPERATING COSTS

Annual costs for on-site operations were reviewed as part of the municipal sludge composting study. As shown in Table 7, over 75 percent of the annual operating cost for aerated static pile systems is associated with labor, bulking agent purchase and aeration pipe replacement. The annual operating breakdown does not include amortized capital cost for facilities and equipment or costs for sludge transport and general administration.

Annual on-site operating costs (1984) for the Hampton Roads, Columbus and Site II facilities based on current operations are about \$33, \$26, and \$24 per wet metric ton (\$30, \$24, and \$22 per wet ton), respectively. Annual average total solids content of the dewatered sludge processed at the three facilities is about 17 percent. Therefore, corresponding unit costs on a dry-weight basis are \$194, \$153, and \$141 per dry metric ton (\$176, \$141, and \$129 per dry ton). Sludge loadings associated with these unit costs at Hampton Roads, Columbus, and Site II are 43, 104, and 338 wet metric tons (47, 115 and 373 wet tons) per operating day, respectively. The Hampton Roads, Site II, and Columbus facilities are operated 6, 5, and 7 days per week, respectively.

TABLE 7. OPERATING COST BREAKDOWN FOR AERATED STATIC PILE COMPOSTING

On-site operating cost category	Percent on total annual cost*
Labor, including fringe benefits	40 to 50
Bulking agent and aeration piping	22 to 42
Power	1 to 10
Fuel	5
Equipment maintenance	11 to 15
Laboratory and other expenses	1 to 8
Total on-site cost	100

*Range based on three facilities investigated.

Labor costs account for about 40 to 50 percent of the annual operating costs at each facility (Table 7). Hampton Roads operates with 8 on-site personnel, Columbus employs 19, and Site II utilizes 42.

Revenue generated from the sale of finished compost at the facilities investigated ranges from about $1 to over $2 per wet metric ton (<$1 to over $2 per wet ton) based on current operations. All facilities have established marketing programs to increase revenue in the future.

CONVENTIONAL WINDROW OPERATIONS

The conventional windrow composting facility at Los Angeles [2-10] processes anaerobically digested sludge from an adjacent wastewater treatment plant consisting of advanced primary sedimentation using anionic polymers and secondary treatment using pure oxygen. Digested sludge is dewatered by both basket and low-speed scroll-type centrifuges. Total daily production of dewatered sludge at the treatment plant is 1,350 wet Mg/d (1,500 wtpd), with approximately 900 wet Mg/d (1,000 wtpd) hauled to a landfill and approximately 450 wet Mg/d (500 wtpd) processed at the adjacent composting facility.

SITE FEATURES

The Los Angeles composting facility employs an asphalt-paved, 10-ha (25-ac) composting field which is the main active windrow composting area. All operations are performed outdoors. The layout also provides areas for equipment storage, research operations, and expanded composting operations.

Several external amendments are used as a bulking agent in conjunction with recycled finished compost, depending on the nature of the finished compost product desired. The sludge-based products produced for sale at this facility are:

1. Nitrohumus--a general soil amendment product which is 90 percent composted sludge and 10 percent sawdust.

2. Topper--a top dressing (new lawn covering, mulch) product that is 60 percent sawdust and 40 percent composted sludge.

3. Amend--a product recommended for vegetable and flower gardens; it is 75 percent rice hulls and 25 percent composted sludge.

4. Gromulch--an outdoor planting mix which is similar to Topper but contains other proprietary ingredients.

Dewatered sludge from the adjacent treatment plant is conveyed to storage silos, and then from the silos to a truck-loading station. Twelve sludge storage silos have a capacity of 500 wet metric tons (550 wet tons) each, for a total capacity of 6,000 wet metric tons (6,600 wet tons). Sludge is stored in the silos during nighttime hours, over weekends, and during rainy periods when disposal (either composting or landfilling) is not possible.

Normal dry-weather operation calls for all silos to be empty by the end of the day shift on Fridays. By the end of the nighttime shift on Sundays, the silos are at their maximum normal dry-weather storage level. From Monday to Friday, storage levels oscillate downward, reaching zero by Friday afternoon.

One operator from a central control panel can control the withdrawal of sludge from all 12 silos and the operation of conveyor belts from silos to two truck-loading stations. Each truck-loading station requires one operator.

Each truck which hauls sludge from the storage silo to the composting field is loaded with approximately 13.5 Mg (15 tons) of wet cake and then moved to a loading area where amendment (rice hulls, sawdust, and/or finished compost) is added from a stockpile. The amount of material added is measured by the bucket volume of a front-end loader. Loaded trucks are then driven to the 10-ha (25-ac) active windrow compost pad where piles are formed.

SLUDGE CHARACTERISTICS

Characteristics of dewatered sludge processed at the Los Angeles facility are summarized below:

Characteristic	Value, percent
Total solids	22-25
Volatile solids	50
Nitrogen (as N)	2
Phosphorus (as P_2O5)	3
Potassium (as K_2O)	0.1

Dewatered sludge density is about 1,050 kg/m^3 (1,800 lb/cu yd) at the range of total solids concentrations indicated.

WINDROW FORMATION

To form a windrow, trucks loaded with sludge and amendment drive to a compost pad and discharge their load. Unloading time for power ram trailers used is approximately 2 minutes. Combining the sludge and amendment in the trailer prior to transport provides only limited initial mixing; thus, the present method of forming a windrow consists of laying two small windrows side by side as the truck unloads. Each small windrow is first turned with a front-end loader, and then the two are pushed into a single windrow with the front-end loader.

Two front-end loaders are used for mixing, amendment loading, and windrow formation. One has a 3.5-m^3 (4.5-cu yd) bucket, and the other has a 4.5-m^3 (5.75-cu yd) bucket. After the preliminary mix during windrow formation, initial windrow mixing is performed using a conventional mobile composter. The conventional mobile composter has the capacity to turn approximately 6 Mg (7 tons) per minute of wet mixture having a bulk density of 900 to 1,000 kg/m^3 (1,500 to 1,700 lb/cu yd). A schematic of windrow formation operations has been presented previously on Figure 3. The cross-sectional dimensions of a typical windrow at the completion of this stage in the composting process are 1.2 to 1.5 m (4 to 5 ft) high and 4.3 m (14 ft) wide at the base.

ACTIVE WINDROW COMPOSTING

The active composting period takes place over a period varying from 30 to 90 days, depending upon ambient temperatures and drying rates. A typical operation for a 56-day active composting period is shown on Figure 4 and described below. Six small windrows such as those described in the previous section are constructed in the first week. Each windrow is about 250 m (800 ft) long with up to 475 wet metric tons (525 wet tons) of dewatered sludge per windrow. At the end of the second week, the six small windrows are combined into four intermediate size windrows. After the sixth week, the four intermediate windrows are combined into a very large windrow. Internal windrow temperatures are monitored in the very large windrow to demonstrate compliance with the criterion of 15 days at 55 degrees C or greater. A large mobile composter is used to form and turn the large windrow, which minimizes heat loss.

A turning frequency of three turns per week has been found to provide adequate pathogen inactivation without affecting drying. The cross-sectional dimensions of the large windrow are 2 m (7 ft) high and 7 m (23 ft) wide at the base. The large windrow is broken down in the ninth week, and the finished compost is delivered to a private company located next to the composting facility which markets the product.

The large mobile composter has the capacity to turn approximately 10 Mg (11 tons) per minute of wet mixture having a bulk density of 900 to 1,000 kg/m^3 (1,500 to 1,700 lb/cu yd). Both mobile composters are self-propelled machines designed specifically for windrow composting. Each machine straddles the windrow and has a high-speed rotating drum at ground level. The drum has flails which lift the sludge up and over the drum, depositing it behind the machine in windrow form. Turning mixes the wet cake and amendment, increases porosity in the windrow to maintain aerobic conditions, promotes drying of the sludge by exposure to air and sun, and ensures that all of the sludge is subjected to the high internal temperatures of the windrows. Table 8 is a summary of windrow properties for the Los Angeles facility.

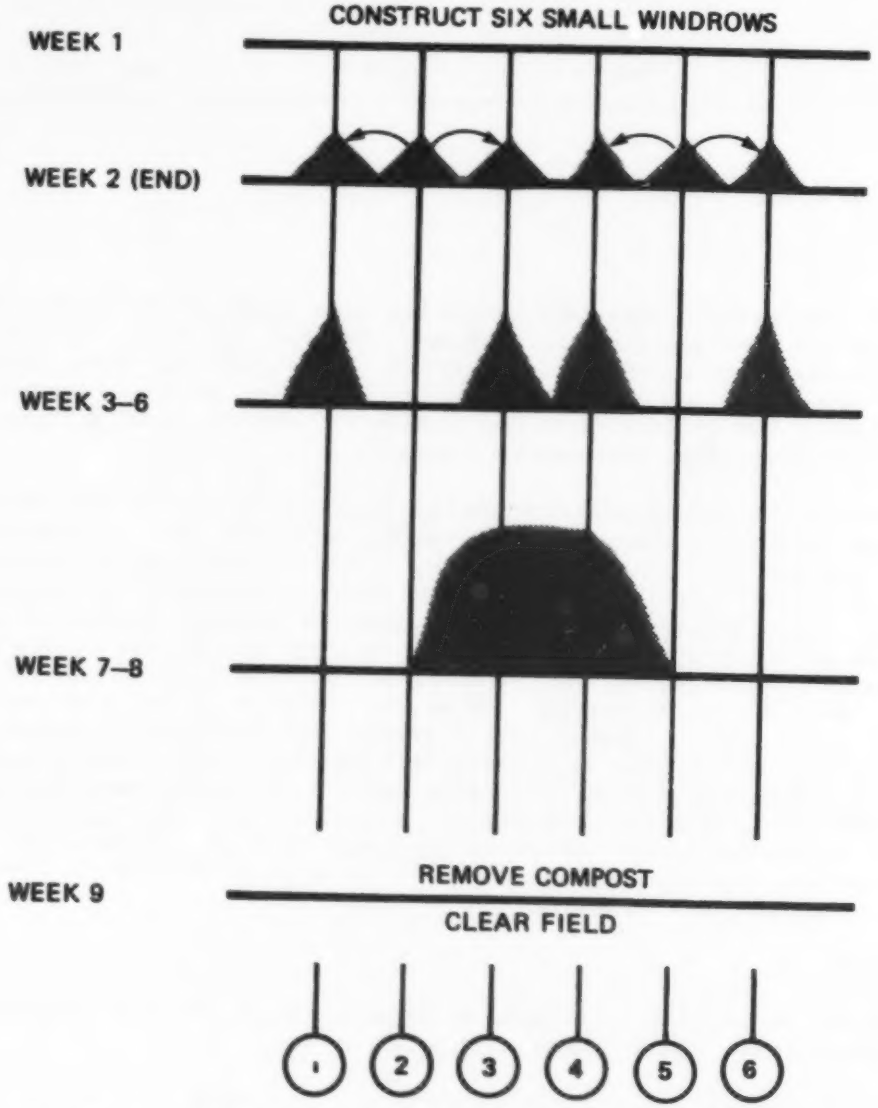

CONSTRUCT SIX SMALL WINDROWS
WEEK 1
WEEK 2 (END)
WEEK 3–6
WEEK 7–8
WEEK 9
REMOVE COMPOST
CLEAR FIELD
1
2
3
4
5
6

FIGURE 4. ACTIVE WINDROW COMPOSTING OPERATIONS AT LOS ANGELES FACILITY

TABLE 8. WINDROW PROPERTIES

Mobile composting machine*	Windrow property		
	Volume, cu yd/ 100 linear ft	Volume, cu yd/ac	Surface to volume ratio, ft/ft
Coventional size	80	1,000	0.83
Conventional size	125	1,800	0.60
Large size	335	3,500	0.32

*See text.

Note: cu yd/100 linear ft x 2.508 = m^3/100 linear m
cu yd/ac x 1.889 = m^3/ha
ft/ft = m/m

Recently, the process described above has been modified to eliminate construction of the intermediate-size windrows. Instead, the large windrows are built directly from three small windrows. Experience has shown that using three, rather than six, small windrows to construct the large windrow gives sufficiently low surface to volume ratios to conserve heat and meet recommended time-temperature performance standards.

Experience at the Los Angeles composting facility has established that for composting to proceed satisfactorily, windrows should have an initial total solids content of at least 40 percent. This is achieved by mixing dewatered cake with previously finished compost material or other amendment. A total solids content of less than 40 percent results in a mixture of low porosity which inhibits oxygen transfer within the windrow. Initial volatile solids content of windrows at the Los Angeles facility is typically about 45 to 50 percent. When the volatile solids have been reduced to 40 to 45 percent, and the total solids have increased to between 60 and 65 percent, the composting process is completed. After the 8-week active windrow composting period, the large windrow is broken down during the ninth week using a front-end loader, and the finished compost is trucked to a stockpile area. After stockpiling, delivery is made to a private company which screens all material for objects 10 mm (3/8 in.) and larger prior to bagging and sale.

PRODUCT QUALITY

The general properties of finished compost from the Los Angeles composting facility are listed below:

Item	Value
Total solids	
Compost with recycled sludge amendment	60-65 percent
Compost with sawdust or rice hull amendment	50-55 percent

Item	Value
Volatile solids	
Compost with recycled sludge amendment	40-45 percent
Bulk densities	
Compost with recycled sludge	770 kg/m^3 (1,300 lb/cu yd)
Compost with sawdust amendment	520 kg/m^3 (875 lb/cu yd)
Compost with rice hull amendment	500 kg/m^3 (850 lb/cu yd)
Carbon to nitrogen ratio	
Compost with recycled sludge	12
Compost with rice hulls	30
Compost with sawdust	135
Particle size	
Median size	1.5 mm (0.06 in.)
90 percent of particles	less than 5 mm (0.20 in.)

Table 9 presents a summary of cadmium, lead, and PCB concentrations detected in compost products from the Los Angeles composting facility. Heavy metal content of the finished compost has increased since 1977. Data prior to 1977 show that finished compost was less contaminated with heavy metals than at present because only large digested sludge particles were captured in the sludge cake with old dewatering equipment used at the treatment plant. Cadmium is associated with smaller digested sludge particles now being captured with new dewatering equipment. Data from the period prior to 1977 show that on a dry-weight basis finished compost cadmium content averaged 26 mg/kg, compared to 50 to 70 mg/kg currently.

TABLE 9. CADMIUM, LEAD AND PCB CONTENT OF WINDROW COMPOST PRODUCTS

Compost product	Concentration, mg/kg dry weight			
	Year	Cadmium	Lead	PCBs
Nitrohumus	1983	60	470	-
	1984	70	510	0.4
Amend	1983	26	250	-
	1984	57	350	0.3
Topper	1983	32	230	-
	1984	47	330	0.3
Recycled compost	1982-83	-	-	1

ODOR CONTROL

Odor measurements at the facility indicate that 83 percent of windrow odor emissions are the result of ambient surface emissions and 17 percent are the result of windrow turnings. This is equivalent to 320,000 odor units per square meter (ou/m^2) (30,000 odor units per square foot (ou/sq ft)) for ambient surface emissions and 65,000 ou/m^2 (6,000 ou/sq ft) for the windrow turnings. These values are based on a 40-day active windrow composting period with 20 turning cycles.

Ambient surface emissions from a windrow decrease significantly as the active compost cycle progresses. This is evident from the chart presented on Figure 5. Emissions are also the greatest immediately after windrow turning, as shown on Figure 6.

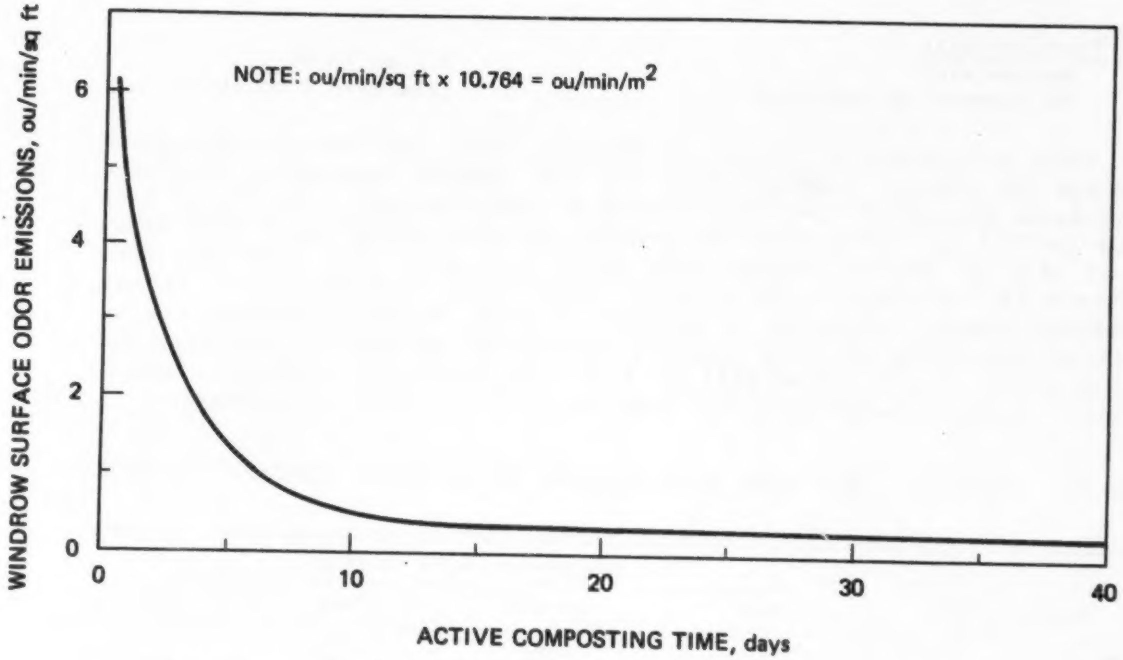


FIGURE 5. AVERAGE WINDROW SURFACE ODOR EMISSIONS DURING A COMPOST CYCLE

Experience at the Los Angeles composting facility has indicated that the best way to control odors and minimize complaints is to limit the size of the composting operation. The maximum amount of sludge which can be composted in the summer without odor complaints is 450 wet Mg/d (500 wtpd). Due to lower productivity in the winter, the annual average is 385 wet Mg/d (425 wtpd), calculated on a 7-day-per-week operation.

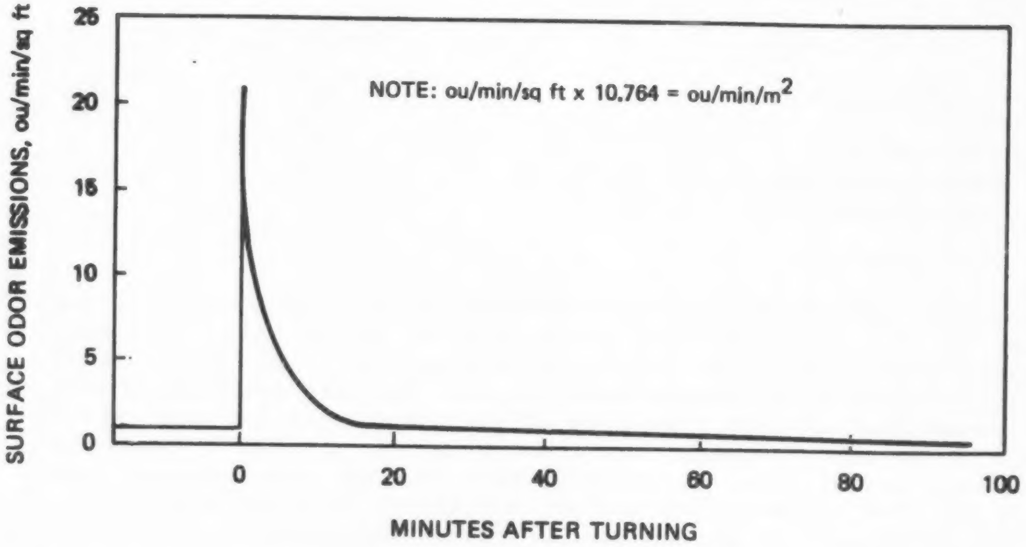


FIGURE 6. SURFACE ODOR EMISSIONS AFTER WINDROW TURNING

Ambient surface emissions from windrow composting cannot be eliminated but are subject to some degree of control based on the type of amendment used. Therefore, when these maximum sludge quantities are exceeded, the odor threshold is exceeded and local residents complain. Even when composted quantities are within these limits, certain meteorological conditions (e.g., inversion layers) can cause odor complaints. Various chemical-masking agents have been tried but have been found to be ineffective.

WINDROW OPERATING COSTS

Operating costs for the latter part of 1984 and the early part of 1985 were reviewed as part of the study. This period reflects current routine operations at the Los Angeles composting facility and specifically excludes a period during the early part of 1984 when current operating procedures were initiated, productivities were low, and supervisory costs were high as personnel were trained in new operations. Composting costs per operating day are presented below:

Item	Dollars per operating day
Equipment capital recovery	1,050
Fuel	475
Maintenance	500
Wages (salary plus fringe benefits) 10 operators plus 1 foreman	2,000
Total costs	4,025

The total cost shown above is equivalent to \$37 per dry metric ton (\$34 per dry ton) or \$9 per wet metric ton (\$8 per wet ton) of sludge composted based on a sludge loading of 450 wet Mg/d (496 wtpd) on an operating day basis at 24 percent total solids. External amendment is used during conventional windrow composting at the Los Angeles facility but is available at no cost. Revenue equivalent to \$4 per dry ton is generated from finished compost sale. Subtracting this from the unit operating cost yields a net cost of about \$33 per dry metric ton (\$30 per dry ton) or \$8 per wet metric ton (\$7 per wet ton) of sludge composted.

Approximately \$300,000 per year is spent on research to better understand the process and to investigate improvements. Labor costs for solids analyses, chemical analyses, and pathogen determinations are approximately \$60,000 per year or \$1.40 per dry metric ton (\$1.30 per dry ton) of sludge cake composted. There is no cost for bulking agents used at the Los Angeles facility.

SUMMARY

Over 40 municipalities in the United States employ composting as a means of treating dewatered sludge prior to ultimate disposal. The majority of these communities employ either the aerated static pile or conventional windrow technique. Raw, aerobically digested and anaerobically digested sludges are processed at average loadings which vary from below one wet Mg/d (1 wtpd) to 450 wet Mg/d (500 wtpd).

Three aerated static pile facilities and one conventional windrow facility were investigated to identify physical characteristics such as site features and equipment, and to define operating procedures. One aerated static pile facility studied processes anaerobically digested sludge, two process raw sludge, and the conventional windrow facility treats anaerobically digested sludge. Nominal dewatered sludge loadings at these facilities vary from 45 to 450 wet Mg/d (50 to 500 wtpd). Current practice at these facilities provides insight into design and operating requirements for applying the municipal sludge composting technologies at other locations.

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